# HYBRIDIZATION STUDIES WITH NUCLEIC ACIDS FROM MURINE PLASMA CELL TUMORS

R. G. Krueger and B. J. McCarthy

Departments of Microbiology, Biochemistry and Genetics
University of Washington
Seattle, Washington 98105

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## SUMMARY

Studies are presented of the hybridization of pulse-labeled RNA from murine myeloma tumors with DNA isolated from the same or different tumors or normal mouse tissues. Rates of reaction, thermal stability and competition experiments all suggest differences among the four different tumor DNAs, a result which may indicate specific amplification of immunoglobulin genes.

## INTRODUCTION

The myeloma tumors of BALB/c mice apparently arise as a result of malignant mutation in a plasma cell or its precursor. Each tumor represents a clone of cells which synthesizes a single homogeneous species of immunoglobulin (1) constituting some 10 to 35% of their total protein (2,3). For this reason, myeloma cells are very attractive subjects for studies concerned with the mechanism of immunoglobulin synthesis and cell differentiation in general. The present communication is concerned with RNA synthesis in myeloma cells and the characterization of pulse-labeled RNA by DNA/RNA hybridization. The results suggest the existence of differences among the DNA's of various myeloma tumors, in those segments of the genome from which some of the pulse-labeled RNA is transcribed.

## MATERIALS AND METHODS

Four different myeloma tumors, supplied through the generosity of Dr. Michael Potter, were maintained as solid tumors by serial transplantation in BALB/c mice. Each of the four tumors synthesize a different antigenically

defined immunoglobulin: MOPC-104E produces IgM globulin, MOPC-173D and ADJ-PC5 produce IgG, while MOPC-46B secretes only light chains (4).

DNA was prepared from nuclei of vigorously growing tumors or BALB/c livers and spleens by standard methods (5).

Tumor RNA was labeled by exposing primary cultures of homogenized tumor in Waymouth's medium to 10 μC/ml of $H^3$-uridine for 30 minutes. Labeled and unlabeled RNA were isolated and purified by standard procedures (5).

DNA filters were prepared by the method of Gillespie and Spiegelman (6) and treated with the preincubation mixture of Denhardt (7). DNA/RNA hybridization was carried out with 5 mm diameter filters containing 5 to 7 μg of DNA in 0.2 ml of 5 X SSC and 50% formamide at 37° C for 16 hours. Amounts of labeled and unlabeled RNA were as specified in figure legends. These particular reaction conditions provide high specificity and minimize loss of DNA from the filters and nucleic acid degradation (8). At the end of the reaction period filters were removed, washed twice with 5 X SSC at the incubation temperature and twice with SSC before drying and counting. Where specified the filters were also treated with RNAse. Thermal stabilities of the DNA/RNA hybrids were determined as described elsewhere (9).

## RESULTS

### Rates of reaction of pulse labeled tumor RNA with DNA.

When pulse-labeled MOPC-173D RNA is incubated with filters containing DNA from the same tumor, a very rapid initial rate of reaction is evident. The effect is less pronounced when DNA from another tumor is used and absent if liver DNA is used (Fig. 1). Similar results have been obtained with RNA isolated from the other three tumors (10). When the same experiments are performed at much higher RNA/DNA ratios, the rapid rate of reaction with filters containing homologous or heterologous tumor DNA or liver DNA are approximately equal (10).

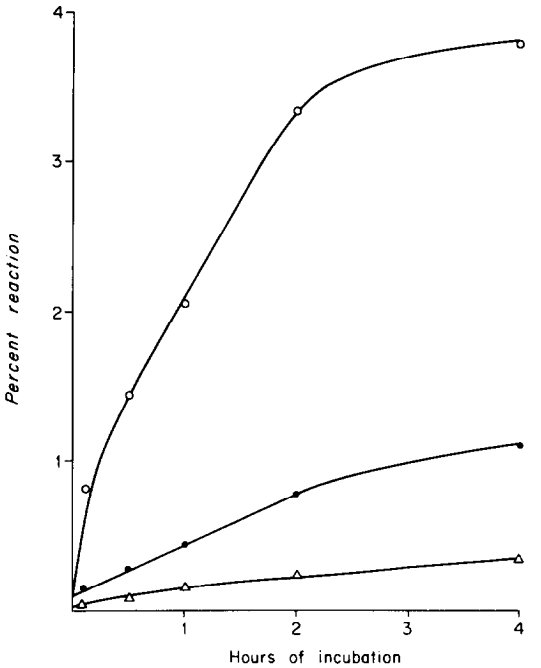


Figure 1: Initial rates of RNA/DNA hybrid formation by 5.5 µg of pulse-labeled MOPC-173D RNA with filters containing 11.0 µg of MOPC-173D DNA (o-o), ADJ-PC5 DNA (•-•) and mouse liver DNA (Δ-Δ) at 37° C in 0.2 ml of 5 x SSC and 50% formamide.

Thermal stability of DNA/RNA hybrids.

Figure 2 illustrates the thermal stability profiles of hybrids formed between ADJ-PC5 RNA and MOPC-173D RNA with ADJ-PC5 DNA. For the homologous combination both the extent of reaction and the mean thermal stability are greater and the hybrids are quite resistant to the action of ribonuclease. When heterologous DNA is used, the thermal stability is much lower and the hybrids are susceptable to ribonuclease digestion. When the same experiments were repeated at higher RNA/DNA ratios the homologous hybrids displayed much lower thermal stabilities similar to those obtained with heterologous DNA. Some of these results are summerized in Table 1. It is evident that the formation of high thermal stability (Tm approximately 80° C), RNAse resistant hybrids occurs only with homologous DNA and at low RNA/DNA ratios. In fact, it can be shown directly that these high stability hybrids result from

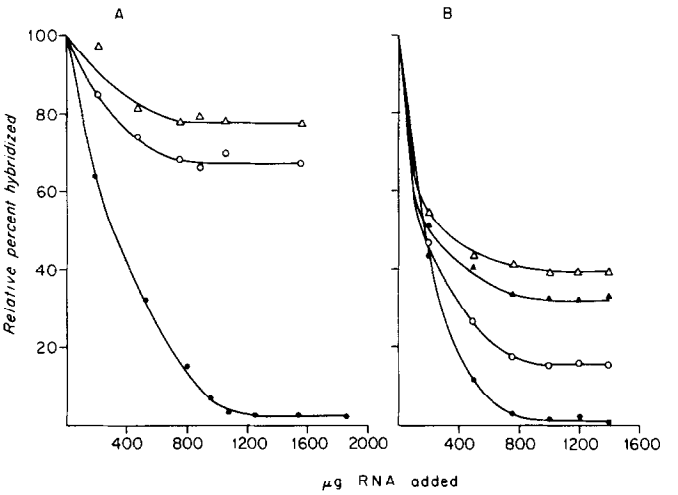


Figure 2: The effect of RNAse on the thermal stability of profiles of RNA/DNA hybrids formed by incubating 5.5 μg of pulse-labeled ADJ-PC5 RNA(A) or MOPC-173 RNA(B) with filters containing 11.0 μg of ADJ-PC5 DNA for 18 hours at 37° C in 0.2 ml of 5 x SSC and 50% formamide. The total hybrid (o-o) was determined by measuring the RNA/DNA complex stable to two washes of 5 x SSC at the reaction temperature. The RNAse resistant hybrid (•-•) remained after treating washed filters, as above, with 10 μg/ml RNAse as described by Gillespie and Spiegleman (5). The filters were removed from the incubation vials, washed and dissociated by heating in 2 ml of 1 x SSC. The temperature of the buffer was raised in steps allowing 15 min for equilibration at each temperature before triplicate filters were removed, washed, dried and counted.

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the rapid initial phase in the reaction (Fig. 1) since at moderate DNA/RNA ratios, the Tm decreases as a function of reaction time (10).

Competition between labeled and unlabeled RNA.

A series of competition experiments has been performed involving various combinations of the four tumor DNAs and the four corresponding RNAs as well as RNA from normal mouse tissues (10). A representative sample of these data is presented here.

When filters of homologous tumor DNA are used, very large differences are discernible between the RNA of ADJ PC5 and RNA isolated from normal liver or spleen (Fig. 3a). These differences are much greater than those observed between RNAs of two normal mouse tissues (9).

Table 1

THERMAL STABILITY OF MYELOMA RNA/DNA HYBRIDS

| RNA | DNA | RNA/DNA Ratios | RNAse Treatment | Percent Reaction | Tm |
|---|---|---|---|---|---|
| PC5 | PC5 | 0.25 | - | 5.76 | 81° C |
| PC5 | PC5 | 0.25 | + | 5.75 | 81 |
| PC5 | PC5 | 0.50 | - | 3.67 | 78 |
| PC5 | PC5 | 0.50 | + | 3.64 | 78 |
| PC5 | 173D | 0.50 | - | 2.75 | 74.5 |
| PC5 | 173D | 0.50 | + | | |
| PC5 | PC5 | 15 | - | 1.20 | 70 |
| PC5 | PC5 | 15 | + | 0.62 | 74 |
| 173D | 173D | 0.25 | - | 4.60 | 81 |
| 173D | 173D | 0.25 | + | 4.60 | 81 |
| 173D | 173D | 0.50 | - | 3.80 | 78 |
| 173D | 173D | 0.50 | + | 3.77 | 78 |
| 173D | PC5 | 0.50 | - | 2.50 | 73.5 |
| 173D | PC5 | 0.50 | + | 1.90 | 77 |
| 173D | 173D | 15 | - | 1.47 | 71 |
| 173D | 173D | 15 | + | 0.71 | 73.5 |
| 173D | PC5 | 15 | - | 1.46 | 66 |
| 173D | PC5 | 15 | + | 0.65 | 70 |

The reaction conditions and determination of the thermal stabilities of the DNA/RNA hybrids are described in the Materials and Methods. The RNA/DNA ratios were varied by adjusting the number of DNA filters in the incubation mixtures.

The same kind of experiment in which unlabeled RNAs from the four tumors were used indicates considerable differences among them. For example, Fig. 3b shows that none of the heterologous tumor RNAs were as effective competitors as homologous ADJ PC5 RNA. It is noticeable, however, that the effectiveness of each competitor is correlated with the type of immunoglobulin produced by each tumor. For example, RNA from the γG producer, MOPC 173D, is more efficient in competition than RNA prepared from MOPC 104E, a γM producer. Similar results were obtained for the RNA isolated from the other three tumors; in each case the effectiveness of heterologous RNA competition is related to the structure of the respective immunoglobulin (10).

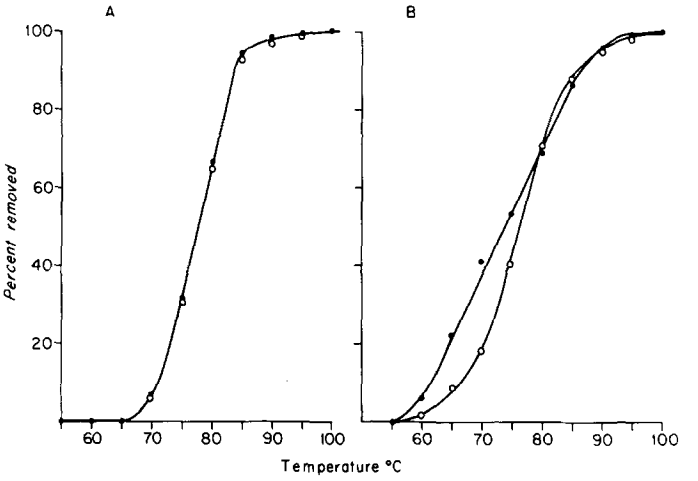


Figure 3: Competition by unlabeled RNA from various sources in the hybridization reaction of pulse-labeled ADJ-PC5 RNA and ADJ-PC5 DNA(A) 7.0 µg of $H^3$-uridine labeled RNA was incubated with 14.0 µg of DNA at 37° C in 0.4 ml of 2 x SSC and 50% formamide in the presence of increasing amounts of unlabeled RNA from ADJ-PC5 (•-•); mouse spleen (o-o) or mouse liver (Δ-Δ). (B) 5.8 µg of $H^3$-uridine labeled RNA was incubated with 11.6 µg of DNA in the presence of increasing amounts of unlabeled RNA from ADJ-PC5 (•-•); MOPC 173D (o-o); MOPC 104E (▲-▲); or MOPC 46B (Δ-Δ). Incubation conditions the same as for (A) except the reaction volume was 0.25 ml.

## DISCUSSION

An earlier publication of Greenberg and Uhr (11) contained some evidence for differences between the genomes of three mouse myeloma tumors. However, the effect was ascribed to the genes for ribosomal RNA. In the present experiments, we have used total pulse-labeled RNA and are therefore unable to ascribe these effects to any particular group of genes or messenger function. Nevertheless, in view of the very large amount of single immunoglobulin produced in these cells, it seems probable that such messenger RNA represents a sizeable fraction of pulse-labeled RNA.

The differences observed among the various DNAs are most readily measurable when hybridization reactions are carried out for short times at low RNA/DNA ratios. This implicates a species of RNA present in high concentration which saturates all the possible binding sites in DNA, complementary or

partially complementary, in a short period of time. Such sites must therefore be multiply present in the genome but still represent only a small fraction of the total DNA. These binding sites appear to exist in much greater numbers in homologous tumor DNA than in heterologous tumor DNA. Moreover these multiple sites must be identical or nearly so by virtue of the very high thermal stability of the hybrids. In general, filter RNA/DNA hybridization assays, which measure only RNA synthesized from the so-called repeated sequences in the mammalian genome (12), give hybrids with thermal stabilities of some 70-75° C depending upon the specificity of the reaction conditions (9). The hybrids of myeloma RNA with homologous DNA formed at low RNA/DNA ratios have Tms as high as 81° C, close to that displayed by hybrids formed by unique sequences of amphibian DNA (13) and well matched hybrids of Bacillus subtilis DNA and RNA, both of which have the same base composition as mouse nucleic acids (9). At higher ratios, when the more slowly reacting RNA species dominates the reaction, the Tm falls to that expected for redundant sequences (Table 1).

Thus the present results together with much more extensive data to be published elsewhere (10) reveal quantitative differences among the DNAs of four myeloma tumors. Obviously it is necessary to fractionate both the RNA and the DNA before unequivocal conclusions are drawn. Nevertheless it seems possible that such differences are associated with the production of myeloma immunoglobulins and reflect somatic amplification of genes implicated in this process.

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