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RecA protein promotes rapid RNA-DNA hybridization in heterogeneous RNA mixtures

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ABSTRACT

The nucleoprotein filament formed by the RecA protein of *Escherichia coli* on single-stranded DNA catalyzes the hybridization of RNA transcripts with single-stranded DNA sequences at 37°C, *in vitro*. RecA protein rapidly promotes hybridization, even when noncomplementary RNA is in a millionfold nucleotide excess over hybridizing RNA, and in a thousandfold nucleotide excess over hybridizing single-stranded DNA. Heterologous double-stranded DNA and RecA-coated noncomplementary single-stranded DNA are also poor competitors of RNA transcripts produced *in vitro*. Since large excesses of noncomplementary RNA fail to inhibit sharply the hybridization reaction by RecA protein under mild, non-degradative conditions, the reaction may be useful in the identification and isolation of transcripts produced *in vivo*.

INTRODUCTION

Experiments described in the preceding paper showed that RecA protein promotes RNA-DNA hybridization by a reaction pathway that is similar to the pairing of single-strands with duplex DNA catalyzed by RecA protein, but differs from the pathway by which RecA protein reanneals complementary single strands.

The role of RecA protein in RNA-DNA hybridization is not attributable to non-specific aggregation of the reactants, but rather is more specific (1). That case is further strengthened here by observations on the ability of RecA protein to promote hybridization in complex mixtures of nucleic acids.

As RNA-DNA hybridization promoted by RecA protein is remarkably resistant to inhibition by other forms of nucleic acid, the enzymological protocol offers practical prospects for facilitating the cloning of protein-encoding sequences from complex genomes, and for readily identifying rare mRNA molecules in heterogeneous RNA mixtures.

MATERIALS AND METHODS

Materials

RecA protein was purified essentially as described (2). In control reactions where RecA protein was omitted, RecA protein dialysis buffer (buffer R composition: 50 mM Tris-HCl pH 7.5, 5 mM DTT, 0.3 mM EDTA and 10% glycerol) was substituted. T3 and T7 RNA polymerases were purchased from Promega and Boehringer Mannheim. Dithiothreitol (DTT) was from Promega and diethylpyrocarbonate from Sigma. Proteinase K from Boehringer Mannheim, sodium dodecyl sulfate (SDS) from Sigma. 'Molecular Biology Certified' grade agarose was purchased from Bio-Rad, Inc. The sources of the nucleotides and of the reaction buffers were as described (1).

DNA sources

Single-stranded DNA constructs used in the hybridization experiments were prepared, as described (1). Of the three constructs prepared, only constructs D and I were used in the experiments described here. The two constructs shared no complementarity in their single-stranded DNA sequences. Large scale preparations of the single-stranded DNA constructs D and I were done as described (3). The region of complementary single-stranded DNA sequence was approximately one-tenth of the total length of the single-stranded DNA construct.

Double-stranded DNA constructs used in the preparation of DNA templates for *in vitro* transcription were prepared, as described (1). Large scale plasmid preparations of the double-stranded constructs were followed, as described (4).

There were two sources of double-stranded DNA substrates used in the competition experiments of Fig. 4C: 1) λ DNA digested with endonuclease BstEII (New England BioLabs), yielding a ladder of 14 fragments of the following sizes: 8454, 7242, 6369, 5686, 4822, 4324, 3675, 2323, 1929, 1371, 1264, 702, 224 and 117; 2) Bluescript vector KS(+) digested with HgiA1 (New England BioLabs), yielding 4 fragments of the following sizes: 1161, 904, 814 and 85.

RNA sources

Yeast total RNA was from Worthington, Inc.; 16S and 23S rRNA were from Boehringer Mannheim and human HeLa total RNA a kind gift from Dr. Alan Weiner. Rabbit globin mRNA was obtained from Bethesda Research Laboratories, and contained at least 80% mRNA.

Unlabeled RNA transcript and uniformly labeled RNA transcript were prepared by *in vitro* transcription protocols (5). RNA transcripts were prepared as described (1). Transcripts

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D:Sal1 and D:EcoRV were generated by T7 RNA polymerase, while transcript I:EcoR1 was generated by T3 RNA polymerase.

RNA and single-stranded DNA substrates which share sequence complementarity are represented by the same alphabetical letter. D:Sal1 RNA designates RNA capable of forming a hybrid with single-stranded DNA-D. The standard reaction conditions were as described (1).

Assays

Agarose gel electrophoresis: Gel electrophoresis was done with 1% agarose gels, which upon drying were subjected to autoradiography. Usually, the gels were run for 2 hours at 120 V and 100 mA. Native unhybridized RNA and RNA in hybrid form were readily distinguishable, as hybridized RNA migrated as a retarded species (6). Direct quantitation was done from the dried gel with a Betascope 603 blot analyzer (Betagen, Waltham, MA).

Nitrocellulose filter assay for hybrid detection: The nitrocellulose filter-based assay was the same as the assay used to measure the homologous pairing of single-stranded DNA and duplex DNA (7). Under the filtration conditions used, single-stranded DNA was quantitatively retained on the nitrocellulose filter, while native unhybridized RNA was not retained. The signal measured was a direct result, therefore, of RNA sequences hybridized to complementary single-stranded DNA sequences (8, 9).

At given time points in a reaction mixture, aliquots were removed, deproteinized as described above, and added to 4.0 ml of 1.5 M NaCl and 0.15 M sodium citrate (10×SSC). The sample was filtered through a nitrocellulose filter (Sartorius, 0.45 μm pore size) at a flow rate of approximately 4.0 ml/10 sec. The tube was rinsed once with another 4 ml of 10×SSC. The filters were air-dried and placed in vials with 5 ml of Ecoscint liquid scintillation fluid. For every filter experiment, a control with noncomplementary substrates was included, to measure background retention of RNA on the filter. Quantitative measures of hybrid formation by the filter assay corresponded closely to those obtained by direct scanning of the gel (see above).

RESULTS

RNA-DNA hybridization promoted by RecA protein

The system

We prepared the plasmid and phage vectors shown in Fig. 1 of the accompanying paper (1). The plasmid vectors served for the production of specific RNA transcripts *in vitro* from T7 or T3 promoters; and the single-stranded phage vectors served for the production of a unique strand of DNA that was complementary to the transcript, but which lacked a complementary DNA strand. RNA and DNA were hybridized under standard conditions in which single-stranded DNA was first preincubated with RecA protein in the presence of ATPγS, a non-hydrolyzable analog of ATP, and 1mM Mg^{2+}, followed by the addition of labeled RNA and more Mg^{2+}, to bring the concentration of the latter to 12 mM. With the concentration of RNA in the nanomolar range and that of DNA in the micromolar range, hybridizing DNA was in large excess. Hybridization was detected by a filter assay in most experiments (see Methods), or by gel electrophoresis in 1% agarose gels run at 120 V for 2 hours. Hybridized and unhybridized RNA species were visualized by autoradiography.

An example of the hybridization reaction is shown in Fig. 1. At a concentration of 10 μM DNA, a strong hybrid band was seen in 30 min when RecA protein was present. Previous studies had shown that label in this band was sensitive to RNase H and that its formation had requirements which were characteristic of pairing reactions promoted by RecA protein (1).

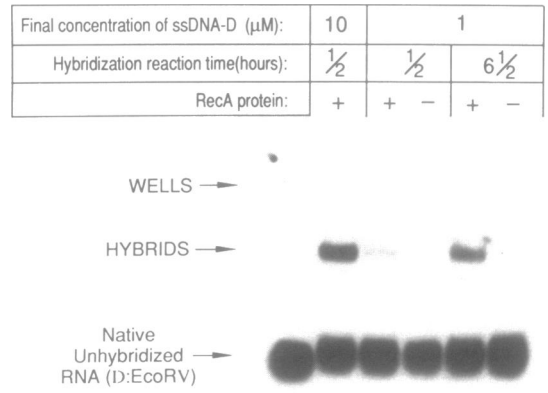

Figure 1. Prolonging the duration of hybridization can compensate for a lower concentration of hybridizing single-stranded DNA. The final nucleotide concentration of single-stranded DNA-D was 10 μM or 1 μM with that of single-stranded DNA-I set at 9 μM in the latter case. ^{32}P-D:EcoRV RNA and unlabeled yeast total RNA, at final concentrations of 1 nM and 1 mM respectively, were pre-mixed and added together to the reaction mixture. Hybridizations were allowed to proceed for 30 minutes or 390 minutes at 37°C, in the presence of RecA protein or protein dialysis buffer. The reactions were done in Hepes buffer at pH 7.6, with ATPγS and a ratio of RecA/single-stranded DNA nucleotides of 1/2. Samples were deproteinized, prior to agarose gel electrophoresis.

Efficient hybridization in heterogeneous mixtures of RNA

Ribosomal RNA

We examined the effect of a competing mixture of 16S and 23S rRNA on our standard hybridization reaction, in the presence of ATPγS (Fig. 2A). This experiment revealed that a one millionfold nucleotide excess of rRNA over the transcript made *in vitro* diminished the yield of hybrid by less than 50%. The addition of so much rRNA also means that total RNA was in a thousandfold excess over hybridizing single-stranded DNA. In this respect, hybridization differs in appearance from the homologous pairing of single strands with duplex DNA. In the latter reaction, efficient pairing requires that single-stranded DNA be approximately equimolar with total duplex DNA (10). This apparent difference between homologous pairing and hybridization promoted by RecA protein presumably arises because of the secondary and tertiary structures of the polynucleotides involved (see Discussion).

Total cellular RNA

The yield of hybrid was diminished by less than 50%, when the concentration of total RNA from human HeLa cells was 5×10^5 times greater than hybridizing transcript RNA, and 500 times greater than hybridizing single-stranded DNA (Fig. 2B). In these experiments, the labeled complementary RNA transcript and unlabeled total HeLa RNA were added at the same time, but the same outcome was seen when HeLa RNA was added 10 minutes prior to the addition of the labeled homologous RNA (data not shown). Hybridization was also insensitive to inhibition by yeast total RNA as shown by the experiment in Fig. 1, in which 1

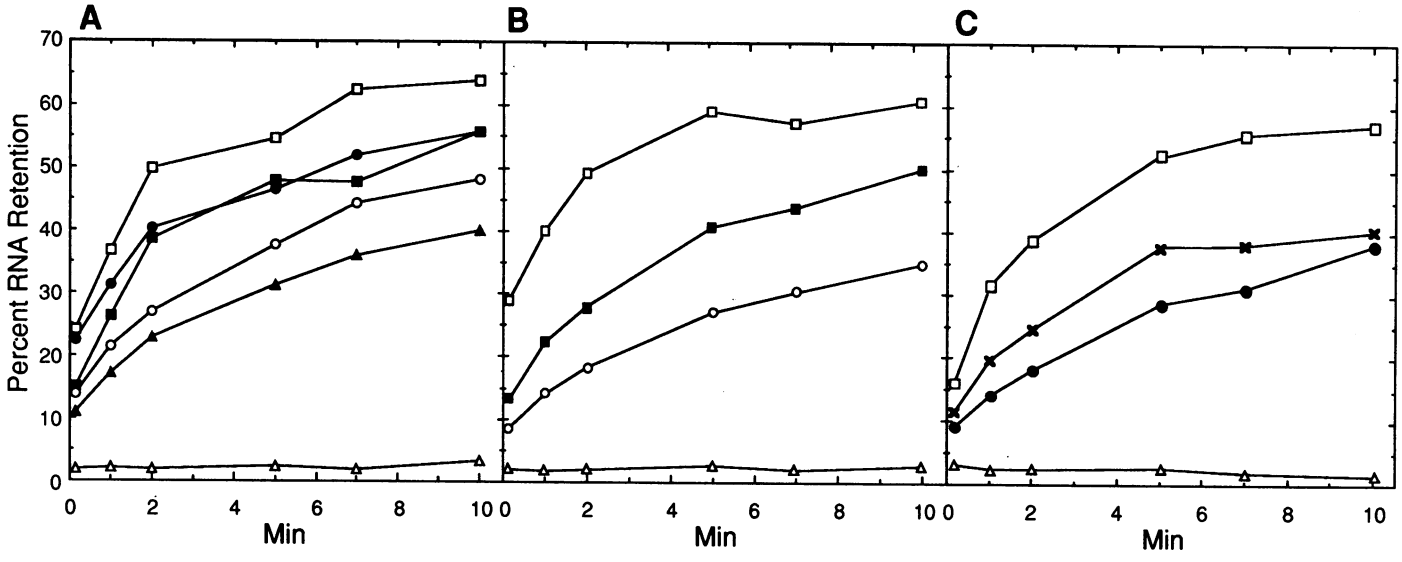

Figure 2. Effect of different competing RNA species on the hybridization reaction. In all three experiments, the standard reaction conditions were followed in Hepes buffer at pH 7.6, with 1 nM ^{32}P-I:EcoR1 RNA pre-mixed with competing unlabeled RNA substrate in substantial excess. The competition reactions were otherwise performed as standard reactions described in the Materials and Methods, and hybridization scored by the nitrocellulose filter assay. **A)** Effect of 16S and 23S rRNA. The final concentrations of 16S and 23S rRNA were 0 μM (□), 50 μM (●), 100 μM (■), 500 μM (○) and 1 mM (▲). **B)** Effect of competing total human HeLa RNA. Human HeLa total RNA was set at a final concentration of 0 μM (□), 100 μM (■) or 500 μM (○). **C)** Effect of competing rabbit globin mRNA. Rabbit globin mRNA was pre-mixed with labeled I:EcoR1 RNA at a final concentration of 0 μM (□), 20 μM (x) or 50 μM (●). In all three noncomplementary controls (△), single-stranded DNA-D was at 10 μM and rRNA was omitted.

mM total RNA was present in all samples. These observations show that RecA protein can rapidly promote hybridization in the presence of a large excess of heterogeneous RNA.

mRNA

Rabbit globin mRNA was utilized in direct competition experiments with RNA transcript generated *in vitro* . Rabbit globin mRNA in fiftythousandfold nucleotide excess over labeled RNA transcript reduced hybridization only about twofold (Fig. 2C). This reduction in yield was comparable to those observed with a millionfold nucleotide excess of rRNA or a 10^5 fold excess of duplex DNA (see below). We observed a similar insensitivity to competition by noncomplementary RNA transcripts made *in vitro* by the same enzymological method that was used to make the labeled complementary RNA (data not shown).

Effects of DNA concentration
Single-stranded DNA

The fraction of labeled complementary RNA that can be incorporated into hybrid form depended upon the concentration of complementary single-stranded DNA in RecA nucleoprotein filaments. In the experiment of Fig. 3A, we kept the total concentration of RecA nucleoprotein filaments constant at 10 μM single-stranded DNA, while varying the fraction of complementary single-stranded DNA. When single-stranded DNA-I was at 10 μM, the concentration of complementary sequences was, thus, about 1 μM, and the yield of hybrid reached 55% in 10 min. In that reaction, noncomplementary single-stranded DNA-D was not present. The apparent rate and the hybrid yield increased steadily as the fraction of complementary DNA rose from 0.01 to 0.1. Furthermore, the experiment in Fig. 1 demonstrates that a tenfold lower concentration of hybridizing single-stranded DNA resulted in a yield comparable to that of the standard reaction, when the duration of the reaction was extended by an equivalent factor.

When the concentration of the single-stranded DNA construct containing the complementary sequences was held constant at 5 μM, and noncomplementary single-stranded DNA was added in fourfold or tenfold excess, there was no reduction in the rate or yield of hybridization (Fig. 3B).

The foregoing observations show that the yield of the reaction is driven by the concentration of complementary sequences, and when this concentration is sufficient the reaction is insensitive to the addition of noncomplementary single-stranded DNA in RecA nucleoprotein filaments.

Double-stranded DNA

Heterologous duplex DNA in twentyfold excess over the single-stranded construct reduced the yield of hybrid only twofold (Fig. 3C). Two preparations of duplex DNA, spanning different ranges of fragment lengths, were used in parallel experiments. Digestion of the DNA vector KS(+) by endonuclease HgiA1 yielded 3 of 4 fragments which were comparable in size to the labeled RNA substrate. Digestion of λ DNA by BstEII yielded fragments that covered a wider range of fragment lengths, most of which were above 1000 nucleotides long. Length of duplex DNA molecules has been identified by others, as a important parameter in their suitability for coaggregation in the presence of Mg^{2+} ions (7). Both preparations of duplex DNA yielded, nonetheless, a comparable effect on the hybridization reaction. Their inability to compete effectively was also observed in the presence of ATP, although it is noteworthy that a final concentration of 20 μM duplex DNA with ATP produced an effect comparable to that observed with 200 μM duplex DNA with ATPγS (11).

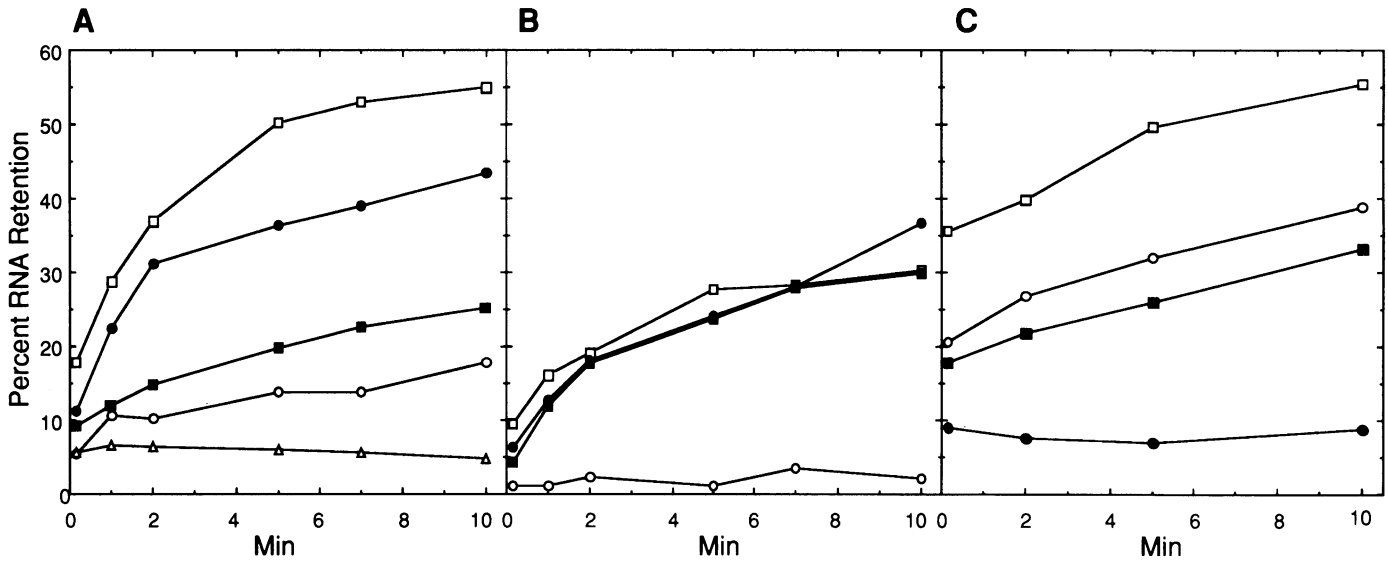

Figure 3. A) Effect of varying the fraction of single-stranded DNA that was complementary to the RNA. The concentration of single-stranded DNA was kept at 10 μM, with complementary single-stranded DNA-I at 10 μM (□), 5 μM (●), 2 μM (■) or 1 μM (○) and the difference adjusted with single-stranded DNA-D. In a control reaction, RecA protein was omitted for the reaction with 2 μM single-stranded DNA-I (△). **B)** Effect of excess noncomplementary single-stranded DNA added to a constant amount of complementary single-stranded DNA. The concentration of single-stranded DNA-I was kept at 5 μM, while single-stranded DNA-D was at 0 μM (□), 20 μM (●) or 50 μM (■). A noncomplementary control was done with single-stranded DNA-D at 5 μM (○). **C)** Effect of fragments of heterologous duplex DNA. There were two sources of duplex DNA fragments: λ digested with BstEII (○), and the Stratagene cloning vector KS(+) digested with HgiA1 (■). In both experiments involving these duplex DNA preparations, the fragments at 200 μM and the ^{32}P-I:EcoR1 RNA at 1 nM were pre-mixed, and simultaneously added to the reaction mixture. The final nucleotide concentration of single-stranded DNA-I was 10 μM. Also depicted are a standard reaction (□) and a control reaction in which RecA protein was omitted (●), with no competing heterologous duplex DNA fragments.

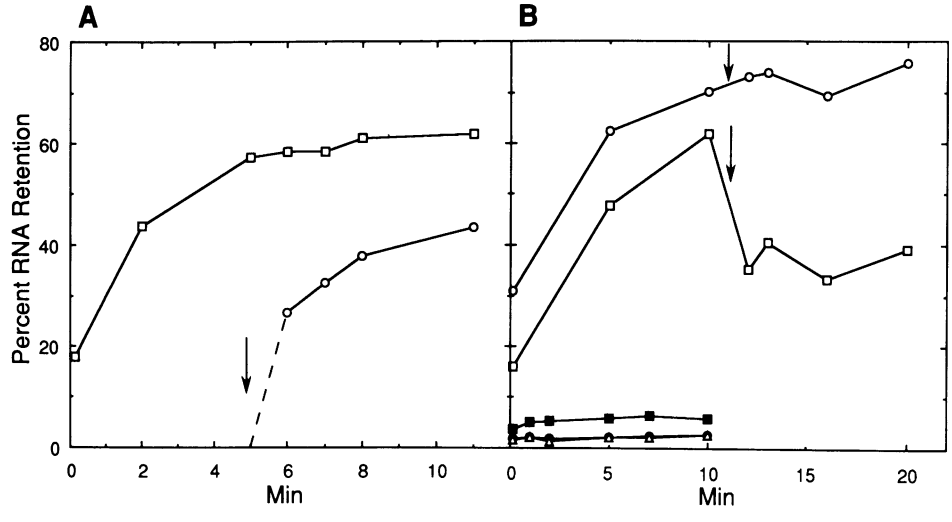

Figure 4. A) The effect of the addition of more RNA, after completion of a first hybridization reaction. This experiment entailed the use of the ^{32}P-labeled (□) and the ^{3}H-labeled I:EcoR1 RNA (○) species. A first hybridization reaction was allowed to proceed with the ^{32}P-labeled RNA for 5 minutes, under standard conditions. The ^{3}H-labeled RNA was then added to the reaction mixture. The percent cpm retained on the nitrocellulose filter was determined for each RNA substrate, after an estimation of the spillover of ^{32}P into the ^{3}H counting channel. The final nucleotide concentration of single-stranded DNA-I was 10 μM, and both RNA species were at a final concentration of 1 nM. The ratio of RecA to single-stranded DNA nucleotides was 1/4; the nucleotide cofactor was ATPγS and the reaction buffer was Hepes at pH 7.6. **B)** Partial displacement of labeled RNA from hybrids by a large excess of unlabeled transcript. The hybridization reaction was done under standard conditions, with single-stranded DNA-I and ^{32}P I:EcoR1 RNA at final concentrations of 10 μM and 1 nM, respectively. Hybridization was monitored 15 sec, 5 min and 10 min into the first reaction. One minute later, unlabeled I:EcoR1 RNA was added at a final concentration of 67 μM (□), and its effect on the prior hybridization of labeled RNA monitored thereafter. Another chase entailed the addition of 67 μM unlabeled noncomplementary D:Sal1 RNA (○). In a competition experiment (■), the labeled and the unlabeled I:EcoR1 RNA were pre-mixed at final concentrations of 1 nM and 50 μM, respectively, and added simultaneously to the reaction mixture. In a reverse chase experiment (●), hybridization was first allowed to proceed with unlabeled I:EcoR1 RNA for 10 minutes, whereupon 11 minutes into the reaction, labeled I:EcoR1 RNA was added to the reaction mixture. In the noncomplementary control, single-stranded DNA-D was at a final concentration of 10 μM (△).

Evidence of Heterogeneity in the Reaction

Since single-stranded DNA was already in large excess in reactions that incorporated only about half of the labeled RNA into hybrid, we explored the possible causes for this incompleteness. An experiment in which we added more RNA after an initial reaction had gone to completion, showed that complementary sites in DNA were indeed in excess: Following the addition of fresh RNA, new hybrid was made (Fig. 4A). This experiment suggested that either part of the population of RNA molecules was unsuitable for forming hybrids or became so during the reaction.

Efforts to detect a change in the RNA during the reaction ruled out some obvious ways in which some of the RNA might be sequestered and become unreactive: No change in the reaction was seen following the preincubation, under the standard conditions of hybridization, of RNA either with RecA protein or with RecA nucleoprotein filaments containing noncomplementary DNA (13).

Evidence of heterogeneity in the RNA substrate and in the hybrid product of the reaction was found in chase experiments (Figs. 4B & 5). When, as a hybridization reaction approached completion, we added a large excess of unlabeled RNA of the same sequence, the label in hybrid promptly fell about 50% (Fig. 4B). Since a similar excess of noncomplementary RNA had no effect on hybrids, the results of the chase cannot be ascribed to general instability of some hybrid molecules in the RecA nucleoprotein filament. Other controls showed, as expected, that all label could be excluded from hybrids if the large excess of unlabeled complementary RNA was added either before or with the labeled RNA (Fig. 4B).

The nature of the observed heterogeneity was further revealed by an experiment in which we added the unlabeled RNA at various times during the reaction (Fig. 5). When the cold chase was added either one or two min following the start of the reaction, further incorporation of label stopped, but hybrid that had already been made did not decrease. A cold chase initiated after 10 min of reaction had the same effect as described above (Fig. 4B): About 40−50% of the label was chased out of hybrid. To determine if the labile component of the hybrids could be stabilized by some slow reaction, we waited 150 min before adding the cold chase; but the loss of labeled hybrid was similar to that seen when the chase was initiated at 10 min into the hybridization reaction.

To look for the most obvious source of heterogeneity in the RNA, namely secondary structure, we heated a sample at 90°C for 2 min and quickly cooled the sample on ice prior to using it for hybridization, but we did not observe any change in the amount of hybrid that was made (11). Intermolecular interactions between the RNA transcripts have not been ruled out, and other data suggest that there may be molecular interactions between the RNA transcripts alone: Ladders of bands were observed for two preparations of RNA transcripts generated *in vitro* . These ladders disappeared upon heating the RNA samples at 90°C for 2 minutes, followed by quick cooling, leaving the fastest migrating band as the only remaining band (11). We observed, furthermore, a non-linear relationship between the concentration of labeled RNA substrate and the absolute counts per minute incorporated into hybrid form, with the latter falling-off as the concentration of transcript was raised (11). If these intermolecular interactions are present, then heat pre-treatment of the RNA prior to the reaction may only temporarily remove them, as they may reoccur in parallel with the hybridization reaction. Interestingly, Nygaard and Hall observed time-dependent decomposition of RNA-DNA hybrids made under thermal annealing conditions (8). The decomposition was also found to be dependent on RNA concentration. Product heterogeneity may thus be a common feature of the enzymological and the thermal annealing reactions.

DISCUSSION

Interactions between the RecA nucleoprotein filament and naked RNA were shown, in the previous paper, to catalyze RNA-DNA hybridization. In this report, we described the effect of competing polynucleotides on the hybridization reaction, finding remarkable resistance to various polynucleotides. The sampling of RNA sequences in complex mixtures of nucleic acids may occur by the rapid diffusion of RNA in aggregates of RecA nucleoprotein filaments. Three noteworthy features of the RecA protein-driven reaction pointed to the rapid diffusion of RNA within the nucleoprotein filaments:

1) Not all RNA molecules were recognized as suitable substrates by the nucleoprotein filament. A mixture of 16S and 23S ribosomal RNA molecules (Fig. 2A) and total RNA mixtures (Figs. 1 & 2B) did not compete effectively with RNA transcripts prepared *in vitro* . We propose that rRNA and tRNA molecules do not compete, because their highly folded structures do not allow their assimilation into the helical groove of the filament. Thus, the RecA nucleoprotein filament serves not only to align complementary polynucleotide sequences, but also to discriminate between the two principal biological classes of RNA: mRNA-like molecules are recognized as suitable substrates, while highly folded RNAs (rRNA and tRNA) are not.

2) Heterologous duplex DNA was a poor competitor of RNA transcripts produced *in vitro* (Fig. 3C).

3) The presence of noncomplementary single-stranded DNA sequences in the nucleoprotein filament did not diminish reaction efficiency, at least when the concentration of hybridizing single-stranded DNA was sufficient to drive the reaction (Fig. 3B).

The notion of rapid diffusion of RNA within the RecA nucleoprotein filaments was further supported by experiments with competing single-stranded polynucleotides. Polynucleotides resembling the *in vitro* transcripts, such as single-stranded DNA fragments (data not shown) and rabbit globin mRNA (Fig. 2C), manifested more obvious inhibition than other forms of RNA or duplex DNA. But even there, inhibition was not dramatic, as a twentyfold nucleotide excess of each over hybridizing single-stranded DNA diminished the standard hybridization yield by only 50%. Furthermore, we found that, while single-stranded fragments were suitable competitors, intact circular molecules competed only marginally (11). This difference may be due to the more limited diffusion of intact single-stranded molecules (10) versus that of fragments.

Both hybridization with RNA and pairing with short double-stranded DNA appear to be stimulated by the formation of a network of nucleoprotein filaments. The formation of networks speeds both reactions presumably by facilitating diffusion, i.e. the confinement of molecules to a more concentrated domain. All hybridization reactions described in this paper were done with ATPγS, under conditions where all RecA-coated single-stranded DNA was aggregated. These conditions were ones in which a nucleoprotein network was already established, and where the interaction with small RNA molecules might be facilitated. We did, indeed, observe that RNA cosedimented with aggregates of nucleoprotein filaments under optimal hybridization conditions

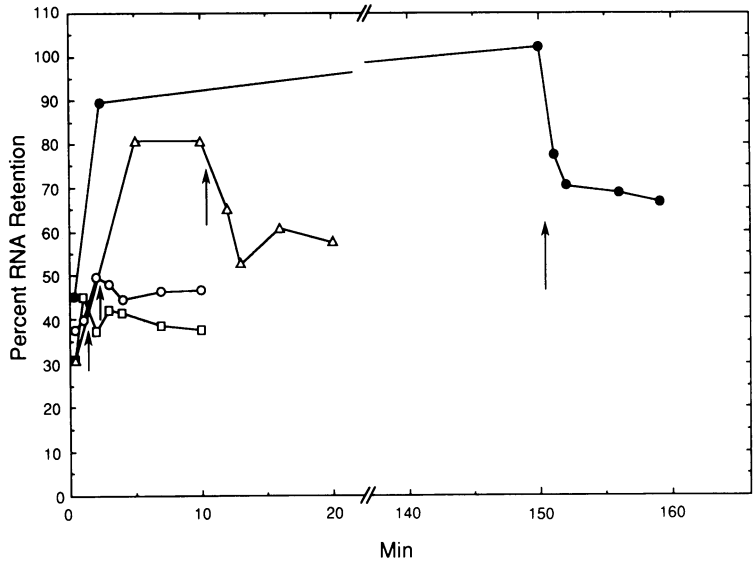

Figure 5. Hybrids formed early in the reaction resist a chase with like-sequence RNA, while those formed later do not. The experiment entailed the addition of cold RNA at different time points into the hybridization reaction. Hybridization reactions were allowed to proceed with 50 μM single-stranded DNA-I and ^{32}P-I:EcoR1 RNA at 1 nM, under the standard conditions described in Materials and Methods. After 1 min (□), 2 min (○), 10 min (△) or 150 min (●) into the reaction, unlabeled I:EcoR1 RNA was added to the ongoing reaction, at a final concentration of 67 μM, and the effect on prior hybridization of the labeled RNA substrate monitored, by the nitrocellulose filter assay.

(1). This process could parallel the RecA-catalyzed pairing of single-stranded DNA with short double-stranded DNA substrates. Earlier, Gonda and Radding had found that homologous pairing with short duplex molecules was inefficient, under different conditions in which RecA nucleoprotein filaments had not aggregated (7). Such a pairing reaction was dramatically stimulated either by the addition of polyamines or long heterologous duplex DNA molecules, both of which produced RecA nucleoprotein networks and consequently enhanced interactions of the filaments with the shorter molecules. Aggregation of the filaments presumably is not required for pairing reactions with long duplex DNA, because its limited diffusion (10) permits the formation of a network. As a consequence of their size and smaller frictional drag, the motion of shorter DNA and of RNA molecules in the RecA nucleoprotein filaments is likely to be more rapid, and thus reactions with both substrates might be stimulated by the prior formation of networks of nucleoprotein filaments.

We observed that the yield of the reaction was dependent on final concentrations of both hybridizing single-stranded DNA (Figs. 1 & 3A) and RNA sequences (Fig. 4A); and prolonged incubation compensated appropriately for a lower concentration of hybridizing single-stranded DNA (Fig. 1). We estimated a second-order rate constant from the initial rates of the reactions shown in Fig. 3A, in the same way as Nygaard and Hall did for thermal hybridization (8). The value calculated from the observed initial rates of hybridization promoted by RecA protein at 37°C, for four different concentrations of DNA, was three orders of magnitude greater than the rate reported for hybridization at 67°C in 0.5 M KCl (8).

In the experiments described in the previous paper, we noted that, in spite of a thousandfold nucleotide excess of hybridizing single-stranded DNA over RNA, not all of the RNA transcript was incorporated into stable hybrids. The detection of two classes of hybrid product of differential stability (Fig. 4C) pointed to heterogeneity in the reaction product. The slowness in the formation of unstable hybrids, relative to the stable class, further suggested that heterogeneity might originate in the RNA substrate itself (Fig. 4C). We have not ruled out secondary structure or intermolecular interactions between RNA transcripts (see Results). The basis for heterogeneity in the reaction product remains unclear. Although some of the RNA substrate does not form stable hybrids, half or more is incorporated, by a reaction that is remarkably resistant to interference by other polynucleotides.

The resistance of RecA-promoted RNA-DNA hybridization to inhibition by other polynucleotides suggests that this reaction may have useful applications in molecular biology. The lack of a requirement for ATP hydrolysis in the hybridization reaction permitted the use of ATPγS throughout this study. ATPγS dramatically reduces the dissociation rate of RecA protein from single-stranded DNA (12, 13), thereby conferring stability on the nucleoprotein filaments. Moreover, the nucleoprotein filaments formed with ATPγS are active in DNA pairing (14), and can be preserved at 4°C for an extended period of time (unpublished observations). The use of ATPγS obviates the need for an ATP regenerating system, involving another enzyme and other reagents, and simplifies the reaction for possible practical use.

We have observed that RNA-DNA hybridization by RecA protein can also be accomplished with oligodeoxynucleotides. RecA protein at 37°C will hybridize an oligonucleotide, 60 nucleotides in length, with its complementary sequences in RNA (unpublished observations). With further development, RecA protein-promoted hybridization could be an attractive alternative to currently existing hybridization protocols, both in solution and on solid supports. It also offers the prospect of recovering intact specific mRNAs from complex mixtures of nucleic acids,

provided one has a single-stranded DNA probe. Finally, where the identification of exons in mammalian genomes is concerned, it offers the promise of circumventing the labor and difficulties of preparing cDNA libraries.

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