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The use of R-looping for structural gene identification and mRNA purification

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ABSTRACT

A method is presented for the purification of mRNAs and the identification of structural gene sequences in recombinant DNA molecules. RNA is hybridized to double-stranded linear DNA such that R-loops are formed between most DNAs and their complementary RNA sequences. These R-loops are purified from unhybridized RNAs by gel filtration chromatography in the presence of a high concentration of salt. The complementary RNAs are released from the R-loops by heating, and are assayed by gel electrophoresis or cell free translation to determine their purity and to identify the proteins for which they code. We have demonstrated that recombinant DNAs containing sequences for abundant or moderately abundant mRNAs of Saccharomyces cerevisiae can be identified by this means.

INTRODUCTION

Recent advances in nucleic acid technology have greatly aided our understanding of the organization and expression of eukaryotic DNA. A number of findings have resulted from detailed studies on viral DNA and cloned segments of eukaryotic DNA. These studies have employed methods which sensitively monitor the genes or gene products of the DNA under study. The presence of a gene, or a portion thereof, can be examined genetically by assessing the ability of purified DNA molecules to complement viral or cellular mutations (1-3). A biochemical approach, initially introduced by Gillespie and Spiegelman (4), involves fixation of the purified DNA to a solid support and subsequent hybridization of the DNA with radioactive RNA (or radioactive DNA transcribed from RNA) isolated from the cell or cells of interest. If the radioactive probe is well characterized and highly purified, successful hybridization to the DNA is usually sufficient to demonstrate sequence homology between the nucleic acid probe and the fixed DNA. If the probe is not homogeneous, unambiguous identification of the eukaryotic DNA requires further characterization of the hybridized radioactive nucleic acid. This is usually accomplished

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by elution of the hybridized RNA from the DNA-solid support and subsequent analysis of the RNA by acrylamide gel electrophoresis, cell-free translation, or some other technique with which it can be identified. Recently, Paterson et al., (5), and Hastie and Held (6) independently published a method which introduces some improvements on previously available procedures for identifying the coding sequences present in viral or recombinant DNA molecules. With this method, there is no fixation of the DNA to a solid support; hybridization of DNA to mRNA is performed in liquid. Since the mRNAs in duplexes are poorly translated in cell-free systems, the ability of a particular DNA species to arrest the translation of specific mRNAs implies complementarity between the DNA and the arrested mRNAs. While this method is of great utility for the analysis of DNAs complementarity to purified or abundant mRNAs, it is somewhat more difficult to detect complementarity to moderately abundant or rare mRNA species whose translation products are not easily detectable. To permit an analysis of complex mRNA populations, we have developed a general method which can detect complementarity between DNA and relatively rare mRNA species. The method exploits the recent and striking observation that RNA/DNA duplexes are more stable than the corresponding DNA/DNA duplexes in the presence of high concentrations of formamide (7-9). Under the appropriate conditions double-stranded DNA molecules hybridize with complementary RNA to form "R-loops". The small fraction of the mRNA present in R-loops can be fractionated on the basis of the physical characteristics of double-stranded DNA and is readily separated from the unhybridized mRNA by gel filtration chromatography. The hybridized mRNA can thus be assayed without the presence of a large background of unhybridized mRNAs. Consequently, complementarity between mRNAs and recombinant DNA can be detected even when these mRNAs - or their protein products - are not readily visible after a one-dimensional fractionation of the total mRNA population. More importantly, perhaps, this method can be used to purify the RNA species complementary to any recombinant DNA.

MATERIALS AND METHODS

Formamide (Matheson, Coleman and Bell) was deionized as described by Maniatis et al. (10) and stored at -80°C. Piperazine-N,-N'-bis 2 ethane sulfonic acid (Pipes) was purchased from the Sigma Chemical Co. Wheat germ was kindly supplied by General Mills, Vallejo, California. [^{35}S] methionine was purchased from Amersham Corporation and [^{32}P] was purchased from New England Nuclear. Wheat germ tRNA (Sigma Chemical Co.) was further purified by phenol extraction and DEAE cellulose column chromatography as described

in Dudock et al. (11). The restriction endonuclease BamHl was purchased from New England Biolabs.

Isolation and Purification of DNA. Recombinant DNA molecules containing the plasmid vector pMB9 with insertions of yeast DNA p(Y), constructed by Petes et al. (12), were isolated from E. coli K12 strain HB101 by standard procedures (12). The supercoiled recombinant DNA molecules pY3-83 and pY11-10 were linearized by digestion with restriction endonuclease BamHl, which cuts each of them once in the vector. Linearized plasmid pPW 311 DNA, a hybrid of pMB9 and sheared Drosophila melanogaster DNA, constructed with A-T tailing like the p(Y) plasmids (12,13), was a kind gift of P. Gergen and P. Wensink. [^{3}H] thymidine labelled pMB9 DNA was kindly provided by J. Morgan. The restriction enzyme-digested DNA molecules were extracted twice with redistilled phenol and twice with $CHCl_3$-isoamyl alcohol (24:1) before use in hybridization reactions.

Preparation of RNA. Total RNA and poly(A)-terminated RNA[1] were isolated from the yeast Saccharomyces cerevisiae as described previously (14). [^{32}P] in vivo labelled total RNA was prepared from 50 ml of yeast grown for one generation (3 hours) at 23°C in the presence of 12.5 mCi of [^{32}P] in YM-1 media (14). Bombyx mori silk fibroin mRNA was generously provided by J. Morrow.

R-Loop Formation. Phenol and chloroform-extracted linear double-stranded DNA was hybridized to total RNA or mRNA in a final volume of 100 µl containing 70%(v/v) deionized formamide, 0.1M Pipes, pH 7.8, 0.01M Na_3 EDTA and 0.4M NaCl (a final $Na^+ \cong$ 0.56 M including contribution from Pipes) (8). Similar results are obtained using the same hybridization solution adjusted to pH 6.8. Incubations were performed by linearly decreasing the temperature from 55°C to 45°C over a four hour period using a Lauda constant temperature bath equipped with a Neslab TP-2 temperature programmer.

Chromatographic Purification of R-Looped RNA. Upon completion of the incubation, 200 µl of column buffer (0.8M NaCl, 0.01M Tris, pH 7.5, 0.001 M EDTA) was added to the 100 µl reaction mixture and applied to a 1 cm x 25 cm agarose A150m, 100-200 mesh column (Bio-Rad Laboratories) equilibrated with column buffer. Fractions of 0.25 ml were collected at a flow rate of 3 mls/hr at room temperature. The excluded and included fractions were each ethanol-precipitated overnight at -20°C with 10 µg of purified wheat germ tRNA carrier. The precipitated nucleic acid was washed twice with 70% ethanol, resuspended in sterile distilled water, heated to 100°C for 1 minute to melt the duplexes, and frozen in a dry ice-ethanol bath.

Cell-Free Translation. RNA samples, including those purified as described above, were translated in a wheat germ extract (15) containing ^{35}S methionine as described by Paterson et al. (5). Often, no stimulation of protein synthesis above endogenous levels was seen with RNA from the excluded fractions of the column since very small quantities of individual mRNAs are present.

Gel Electrophoresis. The products of cell free translation were analyzed by electrophoresis on SDS slab gels containing a linear 10% to 15% gradient of polyacrylamide (16) stabilized by a 5-25% (wt/vol) sucrose gradient. In general, only 10-20% of the included fraction translation product was applied to a lane in order to permit a side-by-side comparison of excluded and included RNA. Two dimensional gels for the analysis of yeast ribosomal proteins were run as described by Gorenstein and Warner (17). All protein gels were subjected to fluorography (18). [^{32}P] labelled yeast RNAs, unlabelled silk fibroin mRNA and yeast RNA were analyzed on SDS-urea gels containing a linear 2.5-5% gradient of polyacrylamide (19). Double-stranded DNA restriction fragments were electrophoresed at 3 V/cm for 16 hours on 1% agarose gels (20).

Biohazard Considerations. All plasmids containing yeast or Drosophila DNA segments were propagated under P2 EK1 containment, in compliance with NIH guidelines for recombinant DNA research.

RESULTS

Many of the hydrodynamic properties of linear recombinant DNA containing R-loops are similar to those of DNA without R-loops and are markedly different from those of single-stranded RNA (21). In particular, double-stranded DNA, with or without R-loops, has a relatively extended conformation in the presence of high ionic strength while single-stranded RNA has a relatively compact conformation under these same conditions. We have exploited these physical properties to separate DNA molecules, including those containing R-loops, from unhybridized RNA by gel filtration chromatography. A gel filtration column demonstrating such a separation is shown in Figure 1.

Linear pMB9 DNA (5.5 kb) and DNA molecules of greater molecular weight chromatograph in the excluded volume and are well separated from yeast 18S and 26S ribosomal RNAs, approximately 2000 and 4000 nucleotides long, respectively. Even silk fibroin mRNA, 16,000 nucleotides long, is partially included and somewhat resolved from the plasmid DNA. Figure 1 also shows that DNA molecules of less than 4.0 kb are partially included. The exclusion limit for double-stranded DNA is thus between 3.5 and 5.5 kb under

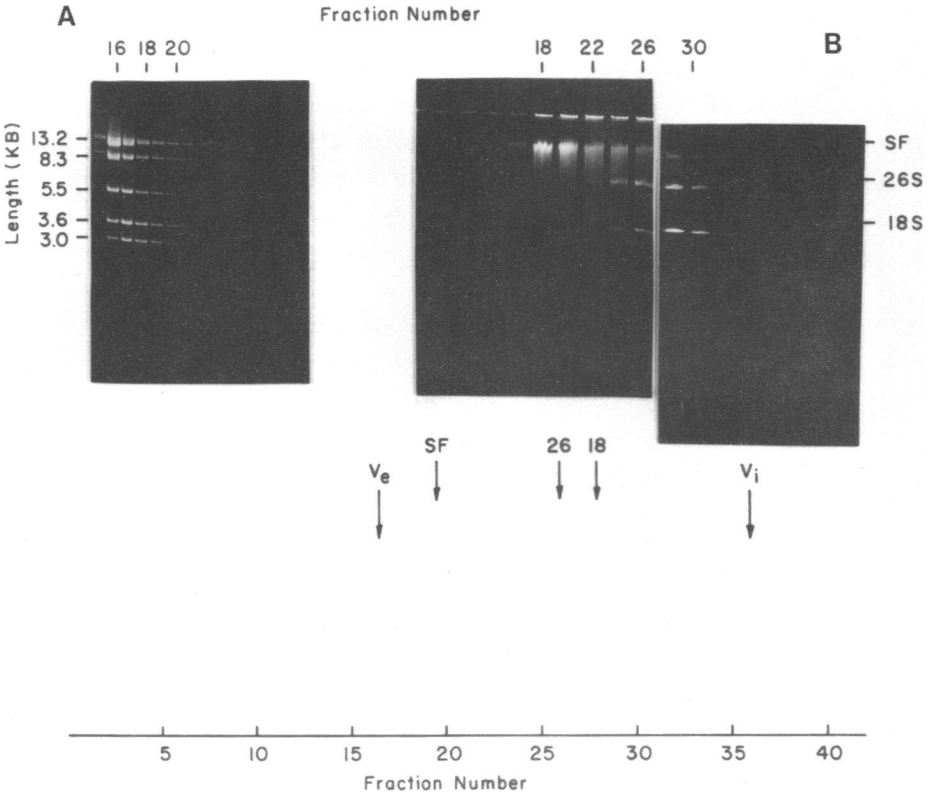

Figure 1. Separation of linear double-stranded DNA molecules from RNA molecules by gel filtration chromatography. Double-stranded DNA restriction fragments, purified silk fibroin mRNA (SF), and yeast 18S and 26S RNAs were incubated at 55°C in 100 µl of hybridization buffer for four hours, chromatographed on an agarose A150m column, and analyzed by gel electrophoresis as described in Materials and Methods. Ve: excluded volume of column as determined by elution position of labelled adenovirus DNA. Vi: included volume, defined by elution position of [^{32}P] phosphate. Inset A, agarose gel of DNA restriction fragments. Inset B, acrylamide gel of RNA.

these conditions. It follows that all recombinant DNA molecules made with the vector pMB9 (5.5 kb) will be entirely excluded.

In order to examine the efficacy of this procedure for the purification of mRNAs complementary to recombinant DNA, we have examined in detail two recombinant DNA molecules (pY3-83 and pY11-10) containing yeast nuclear DNA inserted in the plasmid vector pMB9. These molecules were purified from bacterial strains identified as containing DNA homologous to yeast mRNA by

hybridization with radioactive yeast mRNA (unpublished results). They were thus candidates for molecules containing homology to relatively abundant mRNA sequences and were chosen for the purpose of investigating this method.

An analysis of the result obtained by applying this fractionation procedure to [^{32}P] labelled yeast RNA hybridized to pY3-83 DNA or pY11-10 DNA is shown in Figure 2. A distinct RNA profile is obtained when either DNA is used in the hybridization (lanes 2,3). The RNA species purified with these two DNAs (noted A and B in Figure 2) are distinct from ribosomal RNA and

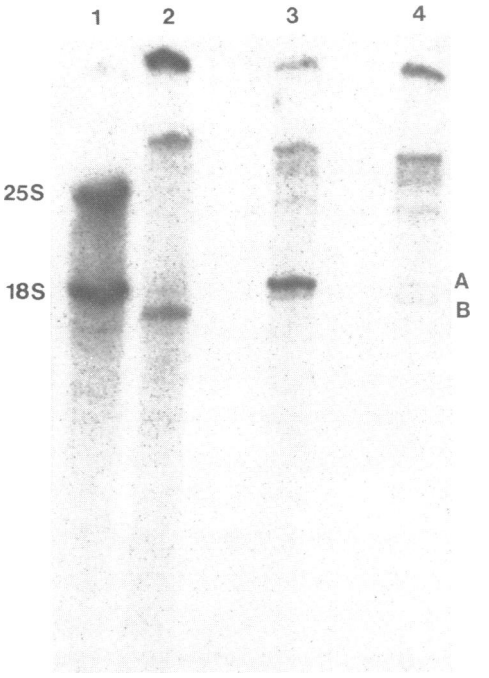

Figure 2. Gel electrophoresis of purified radioactive yeast RNA molecules. Seven micrograms of yeast RNA, labeled in vivo to a specific activity of 10^5 cpm/µg with [^{32}P], were incubated with pY3-83 DNA and pY11-10 DNA and chromatographed on a gel filtration column as described in Materials and Methods, and Figure 1. The nucleic acid in the excluded and included volumes was ethanol precipitated, and aliquots analyzed by electrophoresis on SDS urea gels containing a linear 2.5% to 5% gradient of polyacrylamide. The gel was dried and exposed for one week to Kodak XR5 X-ray film at -80°C using a Dupont Cronex Lightning Plus intensifier screen. Approximately 1000 cpms of [^{32}P] RNA were electrophoresed in each lane:

lane 1: hybridization with pY3-83 DNA, included volume.
lane 2: hybridization with pY3-83 DNA, excluded volume.
lane 3: hybridization with pY11-10 DNA, excluded volume.
lane 4: hybridization with pMB9 DNA, excluded volume.

from each other (data not shown). No discrete yeast RNA species, other than a small amount of ribosomal RNA, are reproducibly found in the excluded volume when the hybridization is performed with pMB9 DNA (lane 4) or with a recombinant DNA molecule containing Drosophila melanogaster DNA pW311 and A-T joints (data not shown). In all cases there is a small amount of radioactive material in the high molecular weight region of the gel. The source of this material is not known but is presumably due to high molecular weight nucleic acid (like fibroin mRNA in Figure 1) which is not well separated from the plasmid DNA by the column chromatography. However, only a small number of discrete RNA species, specific for individual recombinant DNA molecules, are located in the excluded volume of the agarose column.

The same recombinant DNAs have been hybridized to non-radioactive yeast RNA. The RNA found in the excluded volume was assayed by cell-free protein synthesis and subsequent analysis of the protein products (Figure 3). pY3-83 DNA hybridizes to messenger RNA which codes for two polypeptides of molecular weight 51,000 daltons and 48,000 daltons (lane 1, peptides b and c). It is not known whether a single species of mRNA codes for these two proteins or whether they are products of two separate mRNAs. The latter possibility would imply that the mRNA profile in Figure 2, lane 2 is due to more than one mRNA. pY11-10 DNA codes for a single polypeptide of approximately 63,000 daltons (lane 5 and unpublished results). The molecular weights of the 3-83 proteins and the 11-10 protein are consistent with the data in Figure 2 which suggest that the sizes of the mRNAs are somewhat less than and somewhat greater than 2000 nucleotides, respectively. No discrete yeast mRNA is found in the excluded volume of the column when pMB9 DNA, Drosophila DNA or no DNA (data not shown) are incubated with yeast RNA.

Two independent lines of evidence argue that under these conditions, the hybridization goes to completion, or nearly so. Relatively little 3-83 mRNA is available during a second round of hybridization of the RNA in the included volume with pY3-83 DNA (compare lanes 3 and 1 of Figure 3). The presence of translationally active mRNA in the included volumes (lanes 4 and 6) and the availability of a specific mRNA during a second round of hybridization with a different recombinant DNA (11-10, lane 5) argue that most or all of the mRNA survives two rounds of the entire procedure intact. Therefore, the results shown in lanes 3 and 1 of Figure 3 indicate that most or all of the 3-83 mRNA is found in the excluded volume after one round of hybridization with pY3-83 DNA.

A second indication that the hybridization goes to completion is shown

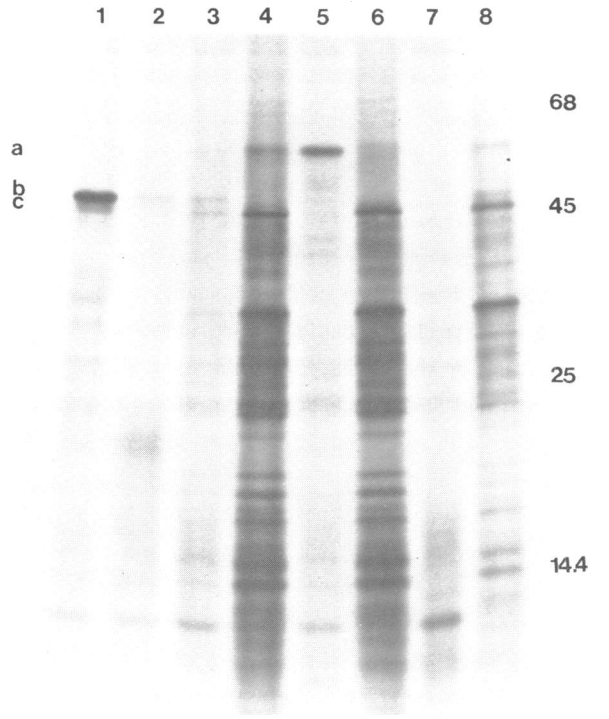

Figure 3. Recycling experiment with hybridized and chromatographed RNA. Seven micrograms of total yeast RNA were hybridized to pY3-83 DNA, and the R-looped RNA was resolved from unhybridized RNA by chromatography as described in Materials and Methods. The hybridized RNA from the excluded volume was ethanol precipitated, resuspended in water, and divided into two aliquots only one of which was heated to melt the RNA:DNA hybrids. The unhybridized RNA in the included volumes was ethanol precipitated, resuspended in hybridization buffer, divided into two aliquots, rehybridized with pY3-83 DNA and with pY11-10 DNA, and chromatographed on two columns. RNA from all of these excluded and included volumes was translated in a cell-free system and the resultant [^{35}S] methionine labelled polypeptides were electrophoresed on an SDS 10-15% gradient polyacrylamide gel as described in Materials and Methods.

lane 1: excluded volume of initial hybridization with pY3-83 DNA, heat melted; major products are bands b and c.
lane 2: excluded volume of initial hybridization with pY3-83 DNA, not heat melted.
lane 3: excluded volume of second hybridization, with pY3-83 DNA.
lane 4: included volume of second hybridization, with pY3-83 DNA.
lane 5: excluded volume of second hybridization, with pY11-10 DNA; major product is band a.
lane 6: included volume of second hybridization with pY11-10 DNA.
lane 7: no added RNA, i.e., endogenous peptides from wheat germ translation.
lane 8: total yeast RNA.

Molecular weight markers, in thousands of daltons, are shown on right.

in lanes 4 and 6 where it can be seen that hybridization with pY11-10 DNA removes most of the mRNA coding for peptide a from the included volume. A similar conclusion is reached for peptide b by comparing lane 8 with either lane 6 or lane 4. Peptide c is difficult to visualize in the translation of total RNA because it migrates very close to a major polypeptide of slighlty lower molecular weight.

Lane 2 demonstrates that the mRNA in the excluded volume is largely unavailable to the translational apparatus in the absence of heating and presumably remains hybridized to the DNA, even after ethanol precipitation. Heating of the re-dissolved RNA and DNA is necessary to permit subsequent synthesis of the appropriate polypeptides (lane 1). This is consistent with previously published data on hybrid-arrested translation (5). The nature of the polypeptides synthesized in the absence of heat melting the R-loops has not been further investigated (lane 2).

We have employed this method to screen a large number of recombinant DNA molecules for the mRNAs with which they share sequence homology. Because of the relatively low background which this technique provides, one can detect mRNAs which do not yield strong protein bands on *in vitro* translation (Figure 4). A pool of four recombinant DNA molecules, all considered possible candidates for containing yeast ribosomal protein gene sequences, were hybridized to yeast poly(A)-RNA and analyzed as described above. At least seven polypeptides result from the translation of the RNA found in the excluded volume. While two of these peptides correspond to major protein products visible in the translation of total yeast RNA, five protein bands are relatively faint. These bands are not visible upon examination of a one-dimensional analysis of the translation products of total yeast RNA and therefore may correspond to polypeptides synthesized from non-abundant mRNAs.

The protein products resulting from the hybridization and fractionation of two such pooled DNA samples (a total of eight different recombinant DNA molecules including the 4 used in Figure 4) were analyzed on a two-dimensional gel system which has been shown to resolve most of the yeast ribosomal proteins (17) (Figure 5). Several protein spots were unambiguously resolved (Figure 5). One of these spots comigrated precisely with the yeast ribosomal protein no. 52 (17) (Figure 5, upper arrow). It follows that at least one of these eight plasmids shares some sequence homology with a ribosomal protein mRNA. The identification and analysis of recombinant DNA molecules containing yeast ribosomal protein genes will be the subject of a subsequent report (Woolford, *et al.*, manuscript in preparation). Another radioactive protein

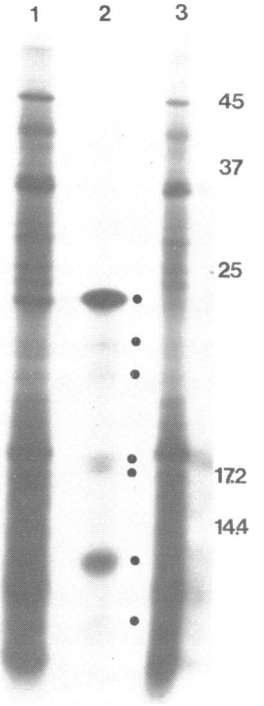

Figure 4. Polypeptides synthesized from yeast RNA complementary to hybrid plasmid DNAs that are candidates for containing ribosomal protein genes. Three micrograms of yeast poly(A) terminated mRNA were hybridized in one reaction to a pool of three micrograms each of four hybrid plasmid DNAs, and chromatographed as described in Materials and Methods. RNA from the excluded and included volumes was translated in a cell-free system, and the resultant [^{35}S] labelled polypeptides were electrophoresed on a 15% polyacrylamide SDS gel, which was fluorographed for three days as described in Figure 2.

lane 1: translation products of one microgram yeast poly(A)-terminated mRNA.

lane 2: translation products of RNA from excluded volume, seven identified protein bands.

lane 3: translation products of RNA from included volume.

Molecular weights of marker proteins are shown on the right.

does not comigrate with any stained ribosomal proteins (lower arrow), but has been shown to comigrate in a number of gel electrophoresis systems with one of the yeast histones. The identification and analysis of recombinant DNA molecules containing yeast histone genes will also be the subject of a subsequent report (Hereford, et al., manuscript in preparation).

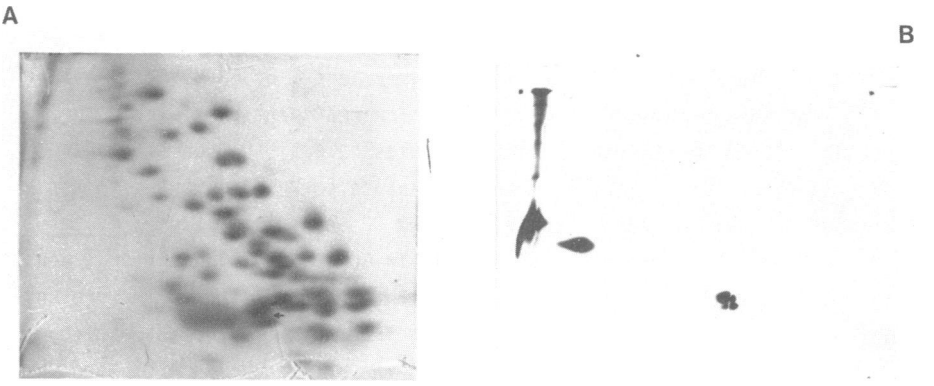

Figure 5. Two dimensional gel electrophoresis of yeast ribosomal protein candidates. (A.) Eight hundred micrograms of total yeast protein were electrophoresed on a two-dimensional gel designed to display ribosomal proteins (17). (B.) Two pools of four hybrid plasmid DNAs containing three micrograms of each DNA were hybridized separately to three micrograms of yeast poly(A)-mRNA, and chromatographed on two columns to isolate the mRNAs complementary to these DNA molecules. These RNAs were translated in a cell-free system containing [^{35}S] methionine, the products pooled, and electrophoresed on one two-dimensional "ribosomal protein gel" (17), which was subjected to fluorography for one week. The spots are bifurcated due to cracking of the gel during drying.

Discussion

The method described in this communication includes an incubation in 70% formamide during which time R-loops form between recombinant DNA and mRNAs which share sequence homology. Because the rate of R-loop formation is a sensitive function of incubation temperature (8,25) which is in turn a function of the particular nucleic acid sequence, efficient reaction conditions should be determined for each recombinant DNA molecule or mRNA under study. In order to permit the analysis of a large number of recombinant molecules without making this determination, we have designed hybridization conditions which appear adequate for the formation of R-loops over a wide range of sequence characteristics.

At constant conditions of ionic strength and formamide concentration, the maximum rate of R-loop formation occurs at the t_{ss} of the specific DNA sequence which is homologous to RNA (8). Under the conditions of R-loop formation used in this report (0.56 M Na^+, 70% formamide), the t_{ss} values of

a number of recombinant DNA molecules containing yeast DNA have been examined in this laboratory (Rosbash et al., manuscript in preparation). When linearized by digestion with a restriction enzyme with a single site in the recombinant DNA molecule, all of these molecules have a t_{ss} of 58-59°C, identical to the t_{ss} of the vector pMB9. Restriction fragments entirely within the eukaryotic portion of the recombinant molecules have t_{ss} values within the range of 45-55°C. It is likely that these values correspond to the t_{ss} of DNA with GC content of approximately 40%, the GC content of S. cerevisiae DNA (22). This range is due to differences in GC content of individual yeast DNA fragments (yeast DNA has an average GC content of 40%) or to the presence of different GC-rich clusters in these yeast fragments. Indeed, the high t_{ss} of the vector pMB9 is most likely due to the presence of one or more GC-rich clusters within the molecule (25). By linearly decreasing the incubation temperature between these two values over a four hour period, most yeast DNA sequences should be within 4°C of their t_{ss} for approximately two hours. At the sequence concentrations used in this report (30 µg/ml of recombinant DNA of maximum complexity 20 kb) and under conditions of DNA excess, this is sufficiently past the $Cot_{1/2}$ such that most or all of the complementary RNA is found in R-loops (vide infra), consistent with previously published data on the rate of R-loop formation (8,25). Since the RNA is routinely assayed after gel filtration chromatography, the R-loops which form must remain largely intact for several hours at room temperature and in aqueous buffers at high ionic strength, consistent with previously published data (8). Although we have observed by electron microscopy large numbers of R-loops in the excluded volume of the agarose column (unpublished observations), the size of these R-loops has not been measured. Consequently, we cannot exclude the possibility that some partial strand displacement of many or all of the molecules may have taken place after transfer of the R-loops to the aqueous buffer.

The minimum length of homology between DNA and RNA necessary to fractionate the RNA into the excluded volume of the agarose column is not known. It is the case, however, that an adenovirus DNA restriction fragment (BamH1 B) containing the leader sequences (23,24) fractionates all of the appropriate adenovirus mRNAs into the excluded volume of the agarose column (R. Ricciardi and B. Roberts, personal communication). Since this DNA contains approximately 215 base pairs of homology with the late adenovirus mRNAs, (23,24), the minimum required length of homology must be less than this value. With regions of homology of less than 100 bases, it is likely that successful fractionation will depend on the precise characteristics of the sequence as well as a more

careful definition of the hybridization and chromatography conditions.

It is important to assess the yield and purification of specific mRNAs that this R-loop procedure provides. The translation data suggest that all, or nearly all, of the complementary mRNA forms R-loops and is present in the excluded volume indicating that the yield is close to 100% (Figure 3). The purification is therefore a function of the initial mRNA abundance and the amount of contaminating mRNA present in the excluded volume. This contamination is relatively minor and manifest by a series of faint bands in the translation products of the RNA found in the excluded volume. This pattern of bands often, but not always, corresponds to the major polypeptide pattern visible in the translation of unfractionated RNA (Figure 3, lane 8) or of RNA in the included volume (Figure 3, lanes 4,6). The contamination is therefore difficult to interpret as well as to measure accurately. From densitometer tracings of the data in Figure 2 it can be estimated that the mRNAs have been purified at least 100-fold relative to ribosomal RNA. The absence of the translation products coded for by major mRNAs in Figure 4, lane 2, and many similar experiments in this laboratory indicate that mRNAs are routinely purified 100-1000 fold relative to non-complementary mRNAs.

The yield and purification of specific mRNAs is related to the concentration of an individual mRNA, as a percentage of the total mRNA (its abundance), which can be detected by this procedure. The protein products of these mRNAs are detectable on one-dimensional acrylamide gels when these products are not visible in the translation of unfractionated mRNA (Figure 4). The translation products of several ribosomal protein mRNAs are also detectable on two-dimensional acrylamide gels (Figure 5, and unpublished results). In addition, β globin is detectable by this assay when five nanograms of globin mRNA is mixed with five micrograms of yeast mRNA, and hybridized to the β globin cDNA plasmid pβG1 (J. Woolford, unpublished results). This reconstructed value of 0.1% of the total mRNA is the approximate level of moderately abundant mRNAs and is likely to be the approximate level of ribosomal protein mRNAs (14). It is likely that mRNAs present at 0.01% of the total mRNA, the approximate concentration rare mRNAs, are detectable by these methods. If so, this method, or some minor modification thereof, will be completely general in defining all of the mRNAs complementary to any recombinant DNA molecule.

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[1] Abbreviations Used

kb	(kilobases), 1000 bases or base pairs in single or double-stranded nucleic acids, respectively
t_{ss}	temperature at which double-stranded DNA is irreversibly converted to single-stranded DNA
Tm	thermal denaturation temperature of DNA (midpoint of helix-coil transition curve)
SDS	sodium dodecyl sulfate
poly(A)	polyadenylic acid
C_0t	product of DNA concentration and time in $\frac{\text{mole sec}}{\text{liter}}$

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