

# Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose

(glyoxal/methylmercuric hydroxide/dimethyl sulfoxide/Southern transfer/dot blot)

PATRICIA S. THOMAS

Fred Hutchinson Cancer Research Center, Division of Genetics, 1124 Columbia Street, Seattle, Washington 98104

Communicated by August H. Doermann, June 20, 1980

**ABSTRACT** A simple and rapid method for transferring RNA from agarose gels to nitrocellulose paper for blot hybridization has been developed. Poly(A)<sup>+</sup> and ribosomal RNAs transfer efficiently to nitrocellulose paper in high salt (3 M NaCl/0.3 M trisodium citrate) after denaturation with glyoxal and 50% (vol/vol) dimethyl sulfoxide. RNA also binds to nitrocellulose after treatment with methylmercuric hydroxide. The method is sensitive: about 50 pg of specific mRNA per band is readily detectable after hybridization with high specific activity probes (10<sup>8</sup> cpm/μg). The RNA is stably bound to the nitrocellulose paper by this procedure, allowing removal of the hybridized probes and rehybridization of the RNA blots without loss of sensitivity. The use of nitrocellulose paper for the analysis of RNA by blot hybridization has several advantages over the use of activated paper (diazobenzoyloxymethyl-paper). The method is simple, inexpensive, reproducible, and sensitive. In addition, denaturation of DNA with glyoxal and dimethyl sulfoxide promotes transfer and retention of small DNAs (100 nucleotides and larger) to nitrocellulose paper. A related method is also described for dotting RNA and DNA directly onto nitrocellulose paper treated with a high concentration of salt; under these conditions denatured DNA of less than 200 nucleotides is retained and hybridizes efficiently.

The technique of Southern (1) enables one to transfer electrophoretically separated DNA fragments to nitrocellulose paper for hybridization with specific radioactive DNA or RNA probes. Although RNA does not generally bind to nitrocellulose, it has been transferred and covalently coupled to activated cellulose paper (diazobenzoyloxymethyl-paper, DBM-paper) according to the method of Alwine *et al.* (2, 3). In our laboratory, the use of activated paper for coupling RNA has been complicated by two major problems: first, about 500 pg of specific RNA per band is just detectable (after several days exposure) after hybridization using high specific activity probes prepared by nick translation (10<sup>8</sup> cpm/μg). Although this sensitivity is similar to that obtained by others with this method (2, 3), we estimate that this is 1-10% of that for detecting specific DNA sequences on nitrocellulose by using similar probes. The increased sensitivity of the nitrocellulose paper is probably a reflection of the fact that nitrocellulose has a higher binding capacity for DNA (about 80 μg/cm<sup>2</sup>) compared to the capacity of most preparations of activated paper for binding RNA or DNA (1-2 μg/cm<sup>2</sup>). Second, we have found that preparation and activation of DBM-paper is expensive, time consuming, and, most importantly, often variable.

Poly(A)<sup>+</sup> RNA is retained on Millipore filters in 0.5 M KCl (4, 5). We therefore decided to investigate whether RNA in general could be bound to nitrocellulose paper and retained during hybridization and stringent washing. Here I describe a rapid and simple method for blot hybridization of denatured

RNA transferred from agarose gels to nitrocellulose paper, using high salt as described by Southern for transfer of DNA to nitrocellulose (1). In our hands the method is easy, reproducible, and about 10-fold more sensitive than the procedure using activated paper. Although, in general, DNA smaller than about 0.5 kilobases does not bind well to nitrocellulose (6), we find that small DNA fragments transfer efficiently to nitrocellulose paper after denaturation with glyoxal and dimethyl sulfoxide (Me<sub>2</sub>SO) and are retained during hybridization. Additionally, RNA or DNA dotted directly onto nitrocellulose pretreated with high salt is retained and hybridizes efficiently.

## MATERIALS AND METHODS

**Materials.** Glyoxal (40% ethanedial in aqueous solution) was obtained from Matheson, Coleman and Bell (Norwood, OH). The glyoxal solution (6 M) was deionized by using a mixed-bed ion-exchange resin (Bio-Rad AG 501-X8) until neutral and stored at -20°C in tightly capped tubes. The glyoxal was stable for several months; however, it was not reused after exposure to air. Me<sub>2</sub>SO was analytical grade obtained from Mallinckrodt. Formamide (ACS grade, Eastman) was deionized by using a mixed bed resin (AG-501-X8) and stored at -20°C.

**General Methods.** The preparation of nick-translated DNA probes (specific activity, 2 to 4 × 10<sup>8</sup> cpm/μg) is given by Weinstock *et al.* (7). Restriction endonuclease fragments of phage λ DNA (cI857 S7) digested with *Hind*III and of phage φX174 replicative form DNA (am<sup>3</sup> ts 70) digested with *Hae* III were obtained from New England BioLabs and incubated with bacterial alkaline phosphatase (Bethesda Research Laboratories, Rockville, MD) to remove the 5'-phosphate groups. The 5' termini of the fragments were labeled with [γ-<sup>32</sup>P]ATP (3000 Ci/mmol, New England Nuclear; 1 Ci = 3.7 × 10<sup>10</sup> becquerels), using phage T4 polynucleotide kinase (Bethesda Research Laboratories) as described (8). Total [<sup>32</sup>P]RNA (specific activity 5 × 10<sup>6</sup> cpm/μg) was the kind gift of R. Hipskind, prepared as described (9) from a line of *Xenopus laevis* kidney cells labeled for 2.5 days with H<sub>3</sub><sup>32</sup>PO<sub>4</sub>. Erythrocytes were prepared from adult anemic chickens or from 5-day chicken embryos and fractionated into nuclei and cytoplasm (10). RNA was isolated from the cytoplasmic fraction by using sodium dodecyl sulfate and proteinase K (11).

## PROCEDURES

**Transfer of Denatured RNA or DNA Fragments to Nitrocellulose Paper and Hybridization.** 1. *Denaturation.* RNA (or DNA) is denatured essentially as described by McMaster and Carmichael (12, 13). Briefly, RNA (up to 10 μg/8-μl reaction mixture) is incubated in 1 M glyoxal/50% (vol/vol) Me<sub>2</sub>SO/10

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: DBM-paper, diazobenzoyloxymethyl-paper; NaCl/Cit, 0.15 M NaCl/0.015 M trisodium citrate (standard saline citrate); Me<sub>2</sub>SO, dimethyl sulfoxide.

mM sodium phosphate buffer, pH 7.0, at 50°C for 1 hr. The reaction mixture is cooled on ice and 2  $\mu$ l of sample buffer containing 50% (vol/vol) glycerol, 10 mM sodium phosphate buffer at pH 7.0, and bromphenol blue is added. The samples are electrophoresed on horizontal 1.1% agarose gels (3 mm thick and 20 cm long) in 10 mM phosphate buffer, pH 7.0, which covers the gel to a depth of about 3–5 mm. RNA is electrophoresed at 90 V for 6 hr; 9S RNA migrates about 9 cm under these conditions. Constant recirculation of the buffer is required to maintain the pH at 7.0; this is critical because glyoxal readily dissociates from RNA or DNA at pH 8.0 or higher. Alternatively, RNA may be denatured and electrophoresed in the presence of methylmercuric hydroxide and the gels prepared for transfer as described (2).

**2. Transfer.** Glyoxalated RNA (or DNA) is transferred from agarose gels to nitrocellulose by using 3 M NaCl/0.3 M trisodium citrate (20 $\times$  NaCl/Cit), essentially as described for transfer of DNA by Southern (1). After electrophoresis the gel (without prior treatment) is placed over two sheets of Whatman 3 MM paper saturated with 20 $\times$  NaCl/Cit. The nitrocellulose paper (Schleicher and Schuell, BA 85, 0.45- $\mu$ m pore diameter) is wet with H<sub>2</sub>O, equilibrated with 20 $\times$  NaCl/Cit, and laid over the gel, covered with two sheets of Whatman 3 MM paper and a 5- to 7-cm layer of paper towels, a glass plate, and a weight. Transfer of RNA is essentially complete in 12–15 hr. We do not treat the gel with alkali to reduce the size of the RNA in the gel, because treatment of the gel with alkali and neutralization with salt buffers substantially reduces the efficiency of transfer of RNA from the gel to the nitrocellulose paper, particularly for larger RNAs. We also find that presoaking the gel in 20 $\times$  NaCl/Cit or staining the gel with ethidium bromide reduces transfer. We obtain the most efficient transfer if the gel is in low salt (10 mM phosphate) and the transfer buffer is high salt (20 $\times$  NaCl/Cit).

**3. Baking.** The blots are dried under a lamp and baked in a vacuum oven for 2 hr at 80°C. Baking is required for retention of the RNA on the nitrocellulose. This step also effectively reverses glyoxalation of the RNA. The blots are not washed with lower salt before baking, because this removes most of the RNA.

**4. Hybridization.** We have used the following buffers as described by Wahl *et al.* (14) with some modifications. The prehybridization buffer contains 50% (vol/vol) formamide, 5 $\times$  NaCl/Cit, 50 mM sodium phosphate at pH 6.5, sonicated denatured salmon sperm DNA at 250  $\mu$ g/ml, and 0.02% each bovine serum albumin, Ficoll, and polyvinylpyrrolidone. The RNA blots are prehybridized for 8–20 hr at 42°C. The hybridization buffer contains 4 parts of the same buffer and 1 part 50% (wt/vol) dextran sulfate. The nick-translated probes are denatured at 100°C for 5–10 min, cooled, and added to the hybridization buffer, and the blots are hybridized for about 20 hr at 42°C. The RNA blots are washed with four changes of 2 $\times$  NaCl/Cit/0.1% sodium dodecyl sulfate for 5 min each at room temperature and then washed with two changes of 0.1 $\times$  NaCl/Cit/0.1% sodium dodecyl sulfate for 15 min each at 50°C. The blots are exposed to X-ray film at -70°C, using a Kodak intensifying screen (Cronex Hi-plus) after the damp blots have been wrapped in Saran Wrap (Dow).

**5. Rehybridization.** Removal of the hybridized probe is accomplished by washing the blots in 0.1–0.05 $\times$  wash buffer (1 $\times$  wash buffer contains 50 mM Tris-HCl at pH 8.0, 2 mM EDTA, 0.5% sodium pyrophosphate, and 0.02% each of bovine serum albumin, Ficoll, and polyvinyl pyrrolidone) for 1–2 hr at 65°C. The RNA blot is then prehybridized and hybridized with the desired probe.

**Dot blot hybridization.** The pretreated RNA or DNA sam-

ples (see *Results*) are spotted directly onto dry nitrocellulose paper that has been treated with H<sub>2</sub>O, equilibrated with 20 $\times$  NaCl/Cit, and dried under a lamp. Samples are spotted onto the nitrocellulose in a small volume (1–5  $\mu$ l) and dried under a lamp. The nitrocellulose dot blots are baked for 2 hr at 80°C, then prehybridized, hybridized, and washed as described above.

## RESULTS

### Binding and Retention of RNA on Nitrocellulose Paper.

The binding and retention of total [<sup>32</sup>P]RNA from *X. laevis* tissue culture cells was first examined. Three different amounts (0.2, 0.02, and 0.002  $\mu$ g) of the purified RNA were denatured by using glyoxal and Me<sub>2</sub>SO according to the procedure of McMaster and Carmichael (12), fractionated on a 1.1% agarose gel, and covalently transferred to DBM-paper as described (2), or noncovalently transferred to nitrocellulose paper by using 20 $\times$  NaCl/Cit as described in *Procedures*. Fig. 1 shows the pattern of *X. laevis* RNA remaining in the gel (A) or transferred and retained (B) on DBM-paper (lanes 1–3) or nitrocellulose paper (lanes 4–6). Transfer of 0.2  $\mu$ g of *X. laevis* RNA to either DBM- or nitrocellulose paper is more than 90% efficient (the efficiency of transfer was estimated visually from shorter exposure autoradiographs). Pretreatment of the gel with alkali followed by neutralization with 0.2 M salt decreased transfer of RNA to the nitrocellulose paper by about 50% (data not shown). RNA is essentially quantitatively transferred to nitrocellulose in about 12 hr. The nitrocellulose paper appeared to bind the smaller amounts of labeled RNA (Fig. 1B, lane 6) somewhat better than did DBM-paper (Fig. 1B, lane 3), because the 18S and 28S rRNA bands are more clearly visible on the nitrocellulose than on the DBM paper. Not only is the *X. laevis* RNA transferred efficiently to nitrocellulose, it is retained on the nitrocellulose paper through prehybridization, 2 days hybridization, and the stringent washes employed during a typical blot hybridization experiment. In addition, no loss of RNA is detected after washing the blot for 2 hr in low salt buffer (0.1 $\times$  wash buffer) or after treatment for 5 min at 100°C in H<sub>2</sub>O. Thus, RNA appears to be stably bound to nitrocellulose paper by this procedure. Because we have assayed mainly rRNA, it is clear that poly(A) is not needed for retention of RNA on nitrocellulose.

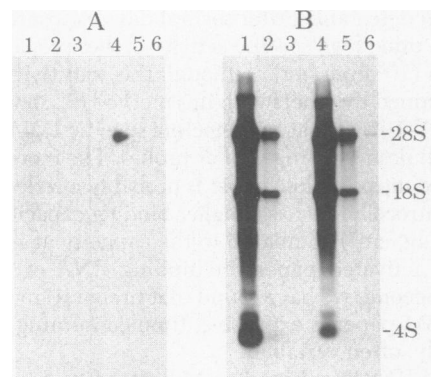


FIG. 1. Transfer of [<sup>32</sup>P]RNA to nitrocellulose and DBM-paper. Total *X. laevis* [<sup>32</sup>P]RNA denatured with glyoxal was fractionated on a 1.1% agarose gel (10<sup>6</sup> cpm, lanes 1 and 4; 10<sup>5</sup> cpm, lanes 2 and 5; 10<sup>4</sup> cpm, lanes 3 and 6) and transferred either to DBM-paper (2) or to nitrocellulose paper according to the procedure described in the text. (A) A 60-min autoradiograph of the gel after transfer to DBM-paper (lanes 1–3) or to nitrocellulose paper (lanes 4–6). (B) A 60-min autoradiograph of the DBM-paper (lanes 1–3) and nitrocellulose paper (lanes 4–6) after transfer, baking, prehybridization (12 hr), hybridization (48 hr), and stringent washing.

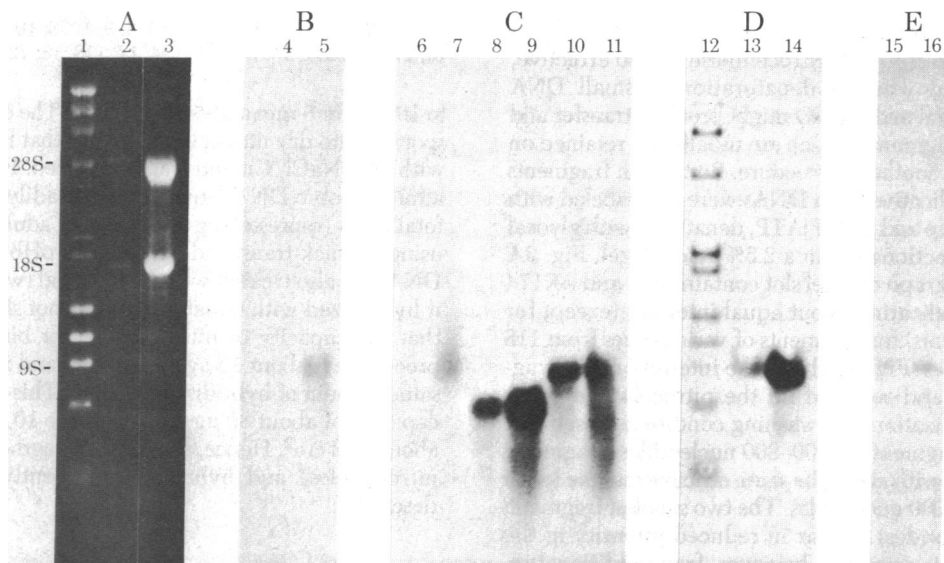
**Conditions Promoting Efficient Transfer and Hybridization of RNA on Nitrocellulose Paper.** Because RNA is stably bound to nitrocellulose even after heating to 100°C for 5 min, we questioned whether glyoxalated RNA was crosslinked to the nitrocellulose or whether the binding was due to the denatured conformation of the RNA. Total cytoplasmic RNA isolated from adult anemic chicken erythrocytes was fractionated on 1.1% agarose gels after various denaturing treatments. The pattern of the ethidium bromide-stained RNA is shown in Fig. 2A. The 9S adult globin mRNA detected by hybridization with the nick-translated β-globin cDNA clone, pHb1001 (15), is shown after transfer to DBM-paper (Fig. 2B) or to nitrocellulose (Fig. 2C and D). It is evident that transfer and hybridization of globin mRNA denatured with Me<sub>2</sub>SO (lanes 8 and 9), with glyoxal plus Me<sub>2</sub>SO (lanes 10 and 11), or with methylmercuric hydroxide (lanes 13 and 14) occurs readily with nitrocellulose paper. The efficiency of hybridization of RNA on nitrocellulose is typically higher than that of RNA on DBM paper (lanes 4 and 5). RNA that has been heated but not treated with denaturing agents appears to bind or hybridize at a much reduced efficiency (lanes 6 and 7). The hybridization signal of 9S globin mRNA detected after treatment of the gel with alkali and neutralization buffer is about 50% of that observed after denaturation with glyoxal and Me<sub>2</sub>SO, Me<sub>2</sub>SO alone, or methylmercuric hydroxide (data not shown). This may reflect renaturation of the RNA during steps prior to transfer. Because all the denaturants tested (except alkali) gave similar quantitative results, it appears that denaturation promotes the binding of RNA to nitrocellulose paper during the transfer process. If a decreased mobility of the globin mRNA reflects the extent of denaturation, then it is likely that full denaturation may not be necessary for mRNA, because the globulin mRNA

treated with Me<sub>2</sub>SO has an increased mobility compared to glyoxal-treated RNA, yet it binds to nitrocellulose and hybridizes efficiently. However, additional data indicate that Me<sub>2</sub>SO is less efficient in promoting transfer of rRNA than of globin mRNA.

It seems puzzling that RNA transferred to DBM-paper hybridized with less efficiency than RNA transferred to nitrocellulose paper, because the transfer of 0.2 μg of labeled rRNA to both papers appeared to be better than 90% efficient (Fig. 1). We would suggest, however, that the greater hybridization efficiency obtained with nitrocellulose was in part due to more efficient transfer of small amounts of RNA; a long exposure of the gel after transfer to DBM- and nitrocellulose papers showed that the transfer of 0.02 and 0.002 μg of *X. laevis* RNA to nitrocellulose paper was essentially complete, whereas detectable amounts of the labeled RNA had not transferred to DBM-paper. Second, the efficiency of hybridization of glyoxalated RNA transferred to DBM-paper was often reduced compared to that of RNA treated with Me<sub>2</sub>SO alone, suggesting that treatment of the gel with alkali before transfer to DBM-paper as described (2) had not completely reversed glyoxalation, hence reducing the hybridization efficiency of the RNA.

Finally, we have found that different lots of nitrocellulose papers (Schleicher and Schuell, Sartorius, and Millipore) bind glyoxalated RNA efficiently. The Schleicher and Schuell and Sartorius papers were more efficient in retaining 4S RNA than Millipore was. Small RNAs have a higher retention on papers with smaller pore size (0.1 or 0.2 μm).

**Sensitivity of Hybridization of RNA on Nitrocellulose Paper.** The data shown in Fig. 2 also suggest that the glyoxal group is probably effectively removed by our standard procedure, because it does not seem to interfere with subsequent



**FIG. 2.** Hybridization of RNA transferred to nitrocellulose and DBM-paper. Total RNA from adult chicken erythrocytes was fractionated on a 1.1% agarose gel after various denaturing treatments and transferred either to DBM-paper (2) or to nitrocellulose paper. The papers were hybridized with the nick-translated cDNA clone pHb1001, which contains sequences specific to chicken adult globin mRNA (15), and exposed for 3 hr, using a Cronex Hi-plus screen at -70°C. (A) Ethidium bromide staining pattern of glyoxal-denatured λ and φX174 fragments (lane 1) and 1 μg (lane 2) or 10 μg (lane 3) of adult erythrocyte RNA. (B) Hybridization signal after transfer to DBM-paper of 1 μg (lane 4) or 10 μg (lane 5) of adult erythrocyte RNA. (C) Hybridization signal of adult erythrocyte RNA transferred to nitrocellulose after heating (50°C, 1 hr), 1 μg (lane 6) and 10 μg (lane 7); after heating in 50% Me<sub>2</sub>SO, 1 μg (lane 8) and 10 μg (lane 9); or after heating in 1 M glyoxal and 50% Me<sub>2</sub>SO, 1 μg (lane 10) and 10 μg (lane 11). (The trailing of 9S mRNA in lane 11 was caused by distortion during transfer at the cut edge of the gel; we find that at least 0.5 cm should be left between the edge of the gel and the last lane.) (D) Hybridization signal of adult erythrocyte RNA electrophoresed on a 1.1% agarose gel in the presence of methylmercuric hydroxide and transferred to nitrocellulose paper, 1 μg (lane 13) and 10 μg (lane 14). Lane 12 shows kinase-labeled λ and φX174 fragments electrophoresed on the same gel. (E) Either 0.05 μg (lane 15) or 0.1 μg (lane 16) of total RNA from embryonic erythrocytes denatured with glyoxal and fractionated on a 1.1% agarose gel, transferred to nitrocellulose, and hybridized with the genome clone λCβG-1, which contains the embryonic and adult β-globin genes (15). Autoradiography was for 8 hr, using a Cronex Hi-plus screen.

hybridization of the RNA on nitrocellulose. Compare the hybridization signal of glyoxalated RNA (lanes 10 and 11) to that of RNA treated with  $\text{Me}_2\text{SO}$  (lanes 8 and 9) or methylmercuric hydroxide (lanes 13 and 14). In our procedure, dissociation of the glyoxal-RNA adduct occurs during baking of the RNA on nitrocellulose at  $80^\circ\text{C}$ . Prehybridization of the blots at pH 6.5 is as effective as prehybridization for 20 hr at pH 8.0, conditions that should be completely effective in removing the glyoxal from the RNA (16).

In the experiments described in Fig. 2, hybridization of the  $\beta$ -globin probe to  $1\ \mu\text{g}$  of total adult erythrocyte RNA, which we estimate to contain about 0.1% adult  $\beta$ -globin mRNA and hence about 1 ng of specific mRNA, is readily detectable in a 3-hr exposure. Fig. 2E shows that embryonic  $\beta$ -globin mRNA is detectable in as little as  $0.05\ \mu\text{g}$  of total erythrocyte RNA from 5-day chicken embryos (lane 15). This represents the detection of about 50 pg of embryonic  $\beta$ -globin mRNA in an 8-hr exposure. We estimate that it should be possible to detect 10 pg of a specific mRNA sequence in about 2 days. This is at least a 20-fold increase in sensitivity over the best we have obtained with RNA bound to DBM-paper. Thus it appears that this technique may have the same high sensitivity for detecting RNA as has been obtained for specific DNAs. We also believe that the sensitivity can be improved another 10-fold (to 1 pg) by maximizing the hybridization conditions and by using probes of even higher specific activity ( $10^9\ \text{cpm}/\mu\text{g}$ ).

The hybridization conditions used give specific hybridization because we detect globin mRNA in as little as  $0.05\ \mu\text{g}$  of total erythrocyte RNA, yet we cannot detect any hybridizable globin RNA in as much as  $5\ \mu\text{g}$  of poly(A)<sup>+</sup> RNA from MSB cells (a line of chicken leukemia cells transformed by Marek's disease virus) or  $5\ \mu\text{g}$  of poly(A)<sup>+</sup> RNA from chicken embryo fibroblasts, two cell types not producing globin RNA.

**Retention and Hybridization of Small DNA Fragments on Nitrocellulose Paper.** Because the binding and retention of glyoxal-denatured RNA on nitrocellulose was so effective, we also investigated whether denaturation of small DNA fragments with glyoxal and  $\text{Me}_2\text{SO}$  might promote transfer and retention of these fragments, which are usually not retained on nitrocellulose by the Southern procedure. Restriction fragments of  $\lambda$  and  $\phi\text{X174}$  replicative form DNAs were end-labeled with polynucleotide kinase and  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ , denatured with glyoxal and  $\text{Me}_2\text{SO}$ , and fractionated on a 2.5% agarose gel. Fig. 3A shows an autoradiograph of a gel slot containing  $\lambda$  and  $\phi\text{X174}$  DNA fragments, indicating about equal intensity (except for comigrating fragments) for fragments of various sizes from 118 to 23,000 nucleotides. Fig. 3B shows the intensity of the fragments transferred and retained on the nitrocellulose paper through the hybridization and washing conditions described. It is evident that fragments of 200–300 nucleotides (fragments 12–16) are retained with about the same efficiency as the larger fragments of 500–2000 nucleotides. The two smallest fragments (72 and 118 nucleotides) appear in reduced intensity in this autoradiograph. It is apparent, however, from additional experiments that these small fragments are also largely retained on nitrocellulose paper. Fig. 3C shows the DNA fragments remaining in the gel after transfer. The glyoxal-denatured fragments between 100 and 2000 nucleotides were efficiently transferred and retained on nitrocellulose paper; the larger DNA fragments (6000–23,000 nucleotides) remained, for the most part, in the 2.5% gel used in this experiment.

We tested hybridization of small DNA bound to nitrocellulose by using nucleosomal DNA of about 140–150 nucleotides isolated from staphylococcal nuclease-digested nuclei and a dot blot hybridization assay (17). The DNA was denatured with 0.3 M NaOH, neutralized, and then further denatured by heating

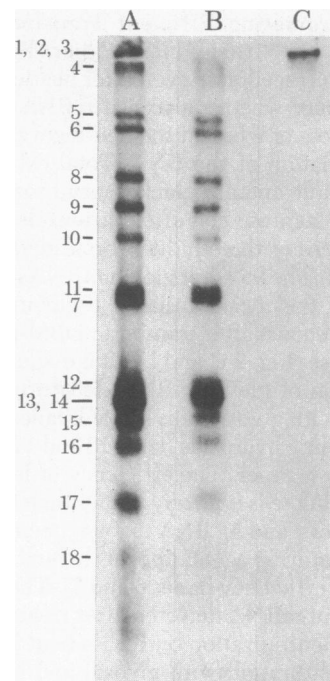


FIG. 3. Transfer of glyoxal-denatured small DNA fragments.  $^{32}\text{P}$ -End-labeled *Hind*III restriction fragments of  $\lambda$  DNA and *Hae* III restriction fragments of  $\phi\text{X174}$  replicative form DNA were denatured with glyoxal, fractionated on a 2.5% agarose gel, and transferred to nitrocellulose. (A) Autoradiograph of  $\lambda$  and  $\phi\text{X174}$  DNA fragments in the gel before transfer. (B) Pattern of fragments transferred to nitrocellulose and retained through mock-hybridization and stringent washing. (C) Autoradiograph of the dried gel after transfer. Autoradiographs were equivalent exposures using a Cronex Hi-plus screen. The sizes of the  $\lambda$  DNA fragments, in nucleotides, are: 1, 23,300; 2, 9500; 3, 6400; 4, 4200; 5, 2200; 6, 1800; 7, 530. The  $\phi\text{X174}$  DNA fragments, in nucleotides, are: 8, 1353; 9, 1078; 10, 872; 11, 603; 12, 310; 13, 278; 14, 271; 15, 234; 16, 194; 17, 118; 18, 72.

to  $100^\circ\text{C}$  for 5 min and cooling on ice. The denatured DNA was spotted onto dry nitrocellulose paper that had been pretreated with  $20\times\ \text{NaCl}/\text{Cit}$  and was hybridized. Fig. 4 shows that the adult  $\beta$ -globin DNA sequences are readily detected in  $1\ \mu\text{g}$  of total DNA (representing about 1 pg of adult  $\beta$ -globin DNA) by using the nick-translated cDNA clone pHb1001 (15). The same DNA was also treated with 1 M glyoxal (without  $\text{Me}_2\text{SO}$ ), and it hybridized with equal efficiency (not shown). We estimate that the capacity of nitrocellulose for binding DNA by this procedure is about  $10\ \mu\text{g}$ , because 10 and  $20\ \mu\text{g}$  give about the same amount of hybridization signal. This represents a binding capacity of about  $80\ \mu\text{g}/\text{cm}^2$ , because  $10\ \mu\text{g}$  was spotted over about  $0.12\ \text{cm}^2$ . Hence, small DNA fragments can be bound to nitrocellulose and hybridized efficiently by the procedure described.

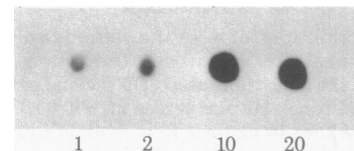


FIG. 4. Dot blot assay of nucleosomal DNA. Nucleosomal DNA of an average length of 140–150 nucleotides was prepared by staphylococcal nuclease digestion of nuclei (10). The DNA was isolated from the monomer fraction, treated with NaOH, neutralized, heated to  $100^\circ\text{C}$  for 5–10 min, and quickly cooled. DNA (1, 2, 10, or  $20\ \mu\text{g}$ ) was spotted in  $4\ \mu\text{l}$  onto the nitrocellulose paper. The dot blot was hybridized with nick-translated DNA from pHb1001 (15) and exposed for 20 hr, using a Cronex Hi-plus screen at  $-70^\circ\text{C}$ .

We also tested binding of RNA to nitrocellulose by using the dot blot assay. We find that  $^{32}\text{P}$ -labeled *X. laevis* RNA is retained on nitrocellulose paper in the dot blot assay described. No loss of labeled RNA is noticeable after treating the RNA blots at the described hybridization and washing conditions (data not shown). In fact, the  $^{32}\text{P}$ -labeled RNA was retained equally well whether the RNA had been treated with 1 M glyoxal or treated with no denaturants. Thus RNA does not need to be denatured to get efficient retention of the RNA in the dot blot assay. This assay is thus very simple and should be quite useful for rapid hybridization of large numbers of RNA samples for determining the relative concentration of a specific RNA sequence.

## DISCUSSION

RNA and small DNA fragments denatured with glyoxal and  $\text{Me}_2\text{SO}$  are transferred essentially quantitatively to nitrocellulose paper and hybridized efficiently by using the standard conditions described in this paper. The technique described by Southern (1) for transfer of large DNA fragments after alkali denaturation is now equally useful for transfer of RNA and small DNA fragments to nitrocellulose paper when the combination of glyoxal and  $\text{Me}_2\text{SO}$  is used for denaturation instead of alkali. Our experiments suggest that the conditions that give the most complete denaturation of RNA (glyoxal plus  $\text{Me}_2\text{SO}$  or methylmercuric hydroxide) are also the most effective in promoting transfer of rRNA and mRNA to nitrocellulose paper. We find, for example, that glyoxal with  $\text{Me}_2\text{SO}$  is more effective than either denaturant used alone for transfer of rRNA, although  $\text{Me}_2\text{SO}$  used alone is sufficient to promote transfer of globin mRNA. Similarly, treatment of RNA with glyoxal,  $\text{Me}_2\text{SO}$ , or methylmercuric hydroxide was more effective in promoting transfer than denaturing the RNA with heat or alkali. It seems probable that RNA denatured by heating or with alkali partially renatures during subsequent steps, thereby reducing the transfer of the RNA under these conditions. Our data suggest that the efficiency of transfer may be largely related to the degree of denaturation of the RNA. The differential effect of  $\text{Me}_2\text{SO}$  on transfer of rRNA and globin mRNA may reflect differences in secondary structure in these RNAs. Although RNA must be denatured for efficient transfer from agarose gels to nitrocellulose paper, it does not need to be denatured to bind efficiently to dry nitrocellulose paper. Thus it seems likely that a denatured conformation is required for RNA to bind to nitrocellulose paper under the equilibrium conditions of transfer. However, once in contact with the nitrocellulose paper by transfer or dotting, denatured or nondenatured RNA is stably bound to the paper through heating to  $80^\circ\text{C}$ . Indeed, if the baking step is omitted, RNA is readily removed from the

paper by washing with  $5\times$  NaCl/Cit. Regardless of the mechanism of binding of RNA to nitrocellulose, it is quite clear from these experiments that RNA can be immobilized on nitrocellulose paper both by transfer from agarose gels and by direct dotting onto nitrocellulose paper. Under the standard conditions of transfer using glyoxal and  $\text{Me}_2\text{SO}$ , we estimate that as little as 10 pg of a specific RNA can be detected in a complex population of RNA species. This represents a sensitivity more than 20-fold better than that reported for covalent transfer of RNA to activated paper (DBM-paper). This method is fast, sensitive, easy, inexpensive, and reproducible.

The author thanks R. Hipskind for the gift of *X. laevis* RNA, A. Larsen for preparation of the nick-translated probes, C. Kane for collaboration with the dot blot experiment, H. Weintraub for encouragement and many helpful comments, and V. Zakian, R. Reeder, M. Groudine, B. Hamkalo, and C. Kane for helpful suggestions on this manuscript. This work was supported by a grant from the National Institutes of Health to Harold Weintraub. P.S.T. is a Postdoctoral Fellow of the American Cancer Society.

1. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503-517.
2. Alwine, J. C., Kemp, D. J. & Stark, G. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5350-5354.
3. Alwine, J. C., Kemp, D. J., Parker, B. A., Reiser, J., Renart, J., Stark, G. R. & Wahl, G. M. (1980) *Methods Enzymol.* **68**, 220-242.
4. Lee, S. V., Mendecki, J. & Brawerman, G. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 1331-1335.
5. Brawerman, G., Mendecki, J. & Lee, S. V. (1972) *Biochemistry* **11**, 637-641.
6. Reiser, J., Renart, J. & Stark, G. R. (1978) *Biochem. Biophys. Res. Commun.* **85**, 1104-1112.
7. Weinstock, R., Sweet, R., Weise, M., Cedar, H. & Axel, R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1299-1303.
8. Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560-564.
9. Khan, M. S. N. & Maden, B. E. H. (1976) *J. Mol. Biol.* **101**, 235-254.
10. Weisbrod, S. & Weintraub, H. (1970) *Proc. Natl. Acad. Sci. USA* **76**, 631-635.
11. McKnight, G. S. (1978) *Cell* **14**, 403-413.
12. McMaster, G. K. & Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 4835-4838.
13. Carmichael, G. G. & McMaster, G. K. (1980) *Methods Enzymol.* **65**, 380-391.
14. Wahl, G. M., Stern, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3683-3687.
15. Stalder, J., Groudine, M., Dodgson, J. B., Engel, J. D. & Weintraub, H. (1980) *Cell* **20**, 451-460.
16. Broude, N. E. & Budowsky, E. I. (1971) *Biochim. Biophys. Acta* **254**, 380-388.
17. Kafatos, F. C., Jones, C. W. & Efstratiadis, A. (1979) *Nucleic Acids Res.* **7**, 1541-1552.