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# Electrophoretic transfer of DNA, RNA and protein onto diazobenzyloxymethyl (DBM) - paper

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ABSTRACT

A method has been developed for the electrophoretic transfer of DNA,RNA,protein and ribonucleoprotein particles from a variety of gels onto diazobenzyloxymethyl (DBM)-paper. Conditions for the electrophoretic transfer of these macromolecules have been optimized to allow for nearly quantitative transfer and covalent coupling. DNA and RNA electrophoretically transferred to DBM-paper retain their ability to hybridize with specific probes. The high efficiency of transfer and the high capacity of DBM-paper for nucleic acids makes possible the sensitive detection of specific nucleotide sequences. Similar efficiency is achieved in electrophoretic transfer and covalent coupling of proteins to DBM-paper. Macromolecules can also be electrophoretically transferred and bound to DBM-paper incapable of covalent bond formation.Their elution from the paper in high salt provides a new and useful preparative method for isolation of DNA,RNA and protein.

## INTRODUCTION

The covalent coupling to diazobenzyloxymethyl (DBM)-paper of discrete species of DNA and RNA,separated by gel electrophoresis, and subsequent hybridization with specific radioactive probes is emerging as a significant advance in the analysis and purification of gene sequences (1-4). Use of DBM-paper avoids difficulties associated with nitrocellulose such as loss of DNA during post-hybridization washes and poor binding of fragments smaller than 0.3 to 0.5 Kb. In addition, DBM-paper has analytical possibilities not offered by nitrocellulose because of its ability to couple covalently with RNA and protein(2, 5).

However, the usefulness of DBM-paper has been limited by the lability of reactive diazonium groups responsible for covalent coupling to macromolecules (2,5). Transferring DNA, RNA and protein from agarose or acrylamide gels to DBM-paper via blotting procedures has resulted in poor transfer and relatively low co-

valent coupling efficiencies (3,6). Only a recent report utilizing in situ DNA cleavage has provided rapid and efficient transfer of DNA molecules (4). Other techniques intended to give faster blotting from gels to DBM-paper have, in our hands, been slow and resulted in losses or chemical alteration of the macromolecules being transferred.

We now describe an electrophoretic transfer procedure which avoids the limitations imposed by blotting techniques. The electrophoretic transfer procedure allows efficient and rapid transfer of intact DNA, RNA, protein and ribonucleoprotein particles from a variety of gels to DBM-paper without any special treatment of the gel or the macromolecules to be transferred.

## MATERIALS AND METHODS

### Preparation of DBM-paper

Nitrobenzyloxymethylpyridinium chloride (NBPC) was synthesized according to the procedure described by Alwine et al.(2) with helpful assistance of Robert Metzenberg. DBM-paper was also prepared as described by Alwine et al.(2).

### Preparation of RNA and polyribosomes

Wild type Neurospora crassa (strain 74-OR8-1a) was grown in 20ml of Fries medium (7) containing 1.0 mM potassium phosphate and 1 mCi of $^{32}PO_4$ (New England Nuclear Corp.). Whole cell RNA was isolated from mid-log phase cultures by homogenizing mycelial pads in 1.8ml of ice cold 1% tri-iso-propyl naphthalene sulfonic acid, 6% p-aminosalicylic acid, and 25 mM ethyleneglycol-bis-(β-amino-ethyl ether)N,N'tetraacetic acid (EGTA) in 50mM Tris-HCl pH 8.1. (Personal communication, R.L.Metzenberg). This step reduced considerably the nuclease digestion of RNA. Two mls of phenol-chloroform-isoamyl alcohol (50:48:2,v/v) were then added to the homogenate and the mixture was shaken vigorously for 1 min. After centrifugation at 8,000xg for 10 min at 4°C the aqueous phase was extracted twice with an equal volume of phenol-chloroform-isoamyl alcohol, and precipitated in ethanol. Bacterial RNA was labeled for 15 min at 30°C with $^{32}PO_4$ in a low phosphate L broth (8) using E.coli strains MRE600 and AB105 (9). Cells were lysed and RNA extracted in SDS and phenol (10). Polyribosomes were prepared from cell lysates (10) and the RNA in these particles

was labeled *in vitro* with cytidine 3',5' diphosphate (5'-$^{32}$P) using T4 RNA ligase (11) generously supplied by S.Rose and O.Uhlenbeck.

Gel electrophoresis of RNA and polyribosomes and transfer to DBM-paper

Electrophoresis of RNA was carried out on 3mm thick verticle slab gels (E-C Apparatus Corp., St.Petersburg, Fl.) containing 2.5% acrylamide,0.5% agarose in Tris/EDTA/borate, pH 8.3 buffer at 200V,0°C for times as indicated in text (12,13). Bacterial polyribosomes were electrophoretically separated in composite gels as described (14). Two-dimensional gel electrophoretic separation of tRNAs was as reported (15). RNA species separated on composite gels were prepared for electrophoretic transfer by incubating the gel in 2 ml of 50 mM sodium phosphate,pH 5.5 per cm$^2$ of gel for 45-60 min at 4°C. Gels containing RNA species larger than 30S were incubated in 3 ml of 25 mM sodium phosphate, pH 5.5 per cm$^2$ of gel for 90 min before transfer. RNA was electrophoretically transferred from gels to freshly prepared DBM-paper in 2-4 liters of phosphate buffer, as above, at 5-10V/cm for 1 to 6 hours at 4°C. Polyribosomes were transferred as described in the text. A schematic diagram of the apparatus used for electrophoretic transfer is shown in Figure 1.

Preparation of DNA

pMF2 DNA (16) was amplified in the presence of chloramphenicol (17) and purified from bacterial lysates by cesium chloride-ethidium bromide buoyant density gradient centrifugation (18).

Gel electrophoresis of DNA and transfer to DBM-paper

Electrophoresis of plasmid DNA restriction fragments was carried out on horizontal slab gels (17x17 cm) containing 0.7 to 1.0% agarose (Seakem LE agarose, Marine Colloids, Inc.) essentially as described by Sharp et al.(19). After electrophoresis portions of the gel were stained with ethidium bromide (0.3μg/ml) for 30 min and then destained in distilled water for 1 hour at 20°C. Before transfer to DBM-paper the DNA in agarose gels was denatured by incubation in 1 ml of 0.5N NaOH per cm$^2$ of gel for 20-30 min at 20°C. The NaOH was decanted and the gel rinsed with distilled water, and then incubated successively in 500, 50 and finally 25 mM sodium

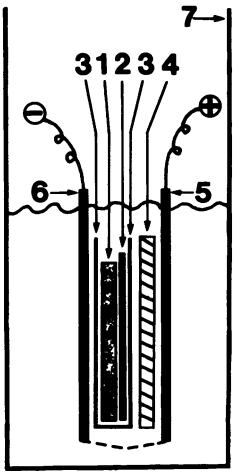


Figure 1. Schematic diagram of apparatus for electrophoretically transferring macromolecules from slab gels to DBM-paper. The apparatus consists of a 23x15cm electrophoretic destainer (E-C Apparatus Corp.) with a porous stainless steel cathode plate (6) and platinum wire anode (5) woven in plastic screen, held by a plexiglas frame and separated by a distance of 2 cm. The gel to be transferred(1) is placed directly on freshly prepared DBM-paper (2) and then wrapped in Whatman 3MM paper (3) presoaked in electrophoresis buffer, care being taken to avoid trapping air bubbles between the gel and paper. The wrapped gel is then placed on a cushion of overlapping nylon screen(4) (Sears) positioned between the anode and the cathode of the destaining apparatus so as to prevent slippage of the gel during electrophoresis. The anode and cathode plates of the destainer,containing the wrapped gel and nylon screen, fit into a plexiglas destainer box (7) (E-C Apparatus Corp.) (17x 24x19 cm) containing sufficient buffer to completely submerge the gel. Current is applied and buffer is circulated from cathode to anode at a rate of 100-200 ml per minute.

phosphate (pH5.5) for 15,15 and 30 min respectively at 4°C in a volume of 1 ml/cm$^2$ of gel. If denaturation was not desired the NaOH and the first sodium phosphate incubation were omitted. DNA was electrophoretically transferred to freshly prepared DBM-paper in 2-4 liters of 25 mM sodium phosphate(pH5.5) at 10V/cm for 4-6 hrs,4°C.

Hybridization of RNA to DBM-paper carrying DNA

Before hybridization, the DBM-paper carrying DNA was incubated for 4 hours at 37°C in 50µl per cm$^2$ of 2xSSC, 0.1% SDS and 1% glycine in a Sears "Seal-N-Save" bag. The preincubation mixture was discarded and replaced by 50µl per cm$^2$ of 2-6xSSC, 0.1% SDS containing 1-10x10$^3$ cpm $^{32}$P— labeled RNA probe per track. Hybridizations were carried out for 16-24 hours at 65°C, after which the paper was washed (37°C) with gentle rocking in 4 changes of hybridization buffer for a total of 90-120 min. The paper was then blotted dry, covered with Saran Wrap and $^{32}$P was detected by autoradiography on Kodak XR-5 X-ray film for 4-24 hours at -70°C using a Dupont Cronex Quanta III intensifying screen.

Hybridization of DNA to DBM-paper carrying RNA

Before hybridization DBM-paper carrying RNA was incubated for

several hours at 20°C or at 4°C to allow maximum covalent bond formation. Pretreatment and hybridization of DBM-paper was as described by Alwine et al.(5). The probe was pMF2 DNA labeled to a specific activity of $1-3x10^6$ cpm/μg by nick translation(20) or, in one experiment,with terminal deoxynucleotidyl transferase (21).

Preparation, electrophoresis and transfer of protein to DBM-paper

Ribosomal proteins labeled with ($^{14}$C)-leucine and separated on one-dimensional polyacrylamide gels accoring to Leboy et al.(22) were electrophoretically transferred at 10V/cm for 2 hrs,4°C, directly onto DBM-paper with 50 mM sodium phosphate,pH 5.5 as the transfer buffer. The DBM-paper carrying proteins was dried, placed directly on Kodak XR-5 film and autoradiographed as above. A similar transfer was made of ribosomal proteins electrophoretically separated in a two dimensional gel system (23) containing 6M urea (kindly supplied by Donald Winkelmann). Urea should be removed from the gel by soaking prior to transfer to avoid swelling of the gel and streaking of the pattern during transfer. Proteins separated in a Tris-glycine-SDS gel system (24) were also electrophoretically transferred to DBM-paper with helpful assistance of Joanna Beachy and Diane Goldman. Prior to transfer the gel was soaked in 50 mM sodium phosphate, pH 6.5,plus 0.1% SDS for 90 min at 20°C to remove Tris and glycine. The gel was then placed on DBM-paper as in Fig 1 and surrounded by a thin plastic sheet with outer dimensions the size of the destainer electrodes and an inner rectangular area (6x 12 cm) cut out to fit around the gel. The plastic sheet was sandwiched between the Whatman 3MM papers or, if the papers were omitted, between two nylon screens. The plastic sheet thus channeled the current entirely through the gel. The protein was electrophoretically transferred from the gel to DBM-paper in 2 liters of 50 mM sodium phosphate, pH 6.5 (no SDS) at 170 mA, about 1-2V/cm for 6 hrs, at 20°C.

## RESULTS

### Electrophoretic transfer of DNA onto DBM-paper

Electrophoretic transfer of labeled DNA restriction fragments (0.91-3.35Kb) from EcoR1 digested pMF2 is a highly efficient process requiring a minimum of manipulation as shown in Table 1.

Less than 2% of the input DNA remained in the gel following 6 hours of electrophoresis at 10V/cm in 25 mM sodium phosphate,

TABLE 1

Covalent coupling efficiency of DBM-paper for electrophoretically transferred, denatured and nondenatured DNA

| | Nondenatured DNA Experiment | | Denatured DNA Experiment | |
|---|---|---|---|---|
| | I | II | I | II |
| cpm remaining in the gel | 467 | 511 | 81 | 45 |
| cpm removed from DBM-paper by washing | 6917 | 5943 | 3401 | 2736 |
| cpm remaining bound to DBM-paper after wash | 24886 | 25013 | 27231 | 20803 |
| percent of DNA coupled to DBM-paper | 77 | 79 | 88 | 88 |

EcoR1 generated restriction fragments (0.91-3.35Kb) of pMF2 were labeled with (alpha $^{32}$P)ATP at 3' OH termini using terminal deoxynucleotidyl transferase (21) and electrophoresed into a 0.7% agarose gel. DNA fragments($10^6$cpm/$\mu$g) in two lanes were denatured and neutralized as described in Methods. Both denatured and nondenatured pMF2 fragments were electrophoretically transferred to freshly prepared DBM-paper in 25 mM sodium phosphate,pH5.5, for 6 hours at 10V/cm and 175mA at 4° C. Following transfer the gels were dissolved in saturated NaI and counted by Cerenkov radiation. After overnight incubation at 4°C the DBM-paper carrying DNA was washed in six changes of 2xSSC,0.1% SDS at 37° C for 24 hours, washes were pooled and counted. The labeled DNA remaining coupled to DBM-paper after washing was hydrolyzed by successive incubations in 1N HCl and 1N NaOH for 60 min each at 37° C. The cpm removed by acid-alkali treatment of DBM-paper carrying DNA were taken as the fraction of counts which were covalently coupled. The covalent coupling efficiency was defined as cpm bound after washing/cpm bound after washing + cpm removed by washing.

pH 5.5. Furthermore DEAE paper positioned anodically from the DBM-paper, and in direct contact with it during electrophoresis, showed no detectable radioactivity, indicating that DNA migration through DBM-paper was negligible. Control experiments showed that DEAE-paper carrying labeled RNA retained it quantitatively under these conditions. Greater than 85% of the denatured DNA and 75% of the nondenatured DNA remained stably bound to DBM-paper through extensive washing (Table 1). Autoradiography of the DBM-paper carrying the labeled DNA showed that, after washing, the four EcoR1-generated restriction fragments of pMF2 with poly A "tails" were transferred and coupled to DBM-paper with comparable efficiencies (data not shown). Poor covalent coupling was

observed with nondenatured EcoR1 and SmaI generated restriction fragments of pMF2 which were labeled with $^{32}P$ by polynucleotide kinase (25). Only 10% of the radioactivity associated with EcoR1 fragments and 1-2% of that associated with SmaI fragments were covalently coupled to DBM-paper following electrophoretic transfer and washing.

Electrophoretic transfer of RNA to DBM-paper

Figure 2 shows that E.coli RNAs ($^{32}P$-labeled) ranging in size from 4S to 23S were completely transferred from a composite gel to DBM-paper in 4 hours at 5V/cm in 50 mM sodium phosphate, pH5.5. As expected the rate of transfer for intact RNAs was size dependent. The 4S and 5S RNAs were completely transferred in 1 hour whereas the larger RNAs required 2 to 4 hours for complete transfer at 5V/cm. In addition complete transfer of a 37-40S RNA of N.crassa from a composite gel as above was achieved in 6 hours by raising the voltage to 10V/cm (data not shown). The covalent coupling efficiency (cpm bound/cpm bound + cpm removed by washing) varied from 60-65% for 4S and 5S RNA to 70-80% for higher molecular weight RNAs.

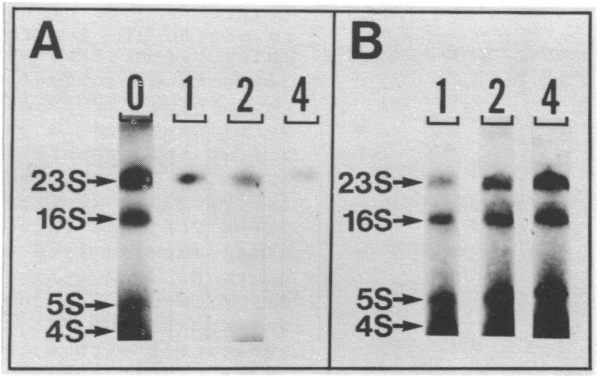


Figure 2. Rate of electrophoretic transfer of RNA from a composite gel to DBM-paper. E.coli RNA, $^{32}P$-labeled, was electrophoresed into a composite gel for 1.5 hours and then prepared for electrophoretic transfer as described in Methods. The RNA was electrophoretically transferred from the gels to DBM-paper for time intervals of 1,2 and 4 hours as described in the text. Following electrophoresis both the gels (A) and the DBM-paper (B) were autoradiographed. Numbers in the slot areas indicate the hours of electrophoresis.

Rate of covalent bond formation of transferred RNA

Alwine et al.(2) have proposed that DNA and RNA initially adhere to DBM-paper by an interaction of positively charged diazonium groups with negatively charged nucleic acids. The ionic interactions are then superseded more slowly by covalent linkages. The rate at which covalent bonds are formed between RNA and reactive diazonium groups following electrophoresis was assesed by electrophoretically transferring $^{32}$P-labeled E.coli 4S RNA from a composite gel to freshly prepared DBM-paper and allowing the DBM-paper carrying RNA to incubate for various lengths of time prior to washing. As shown in Figure 3, RNA-bearing DBM-paper washed immediately after a 90 min electrophoretic transfer had less than 15% of the total 4S RNA covalently attached. However a 2.5 hour incubation at 20°C of DBM-paper carrying RNA was sufficient for maximum covalent bond formation. DBM-paper carrying RNA which was incubated at 20°C following electrophoretic transfer bound 10-15% more 4S RNA covalently than did identical samples incubated at -80°C, suggesting that covalent bond formation does exhibit some activation energy. These results support the suggestion(2) that coupling of macro-

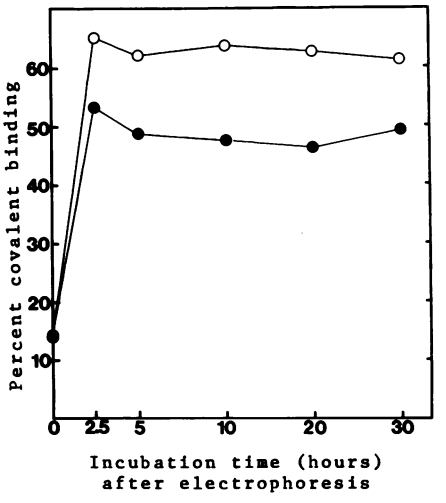


Figure 3. Rate of covalent bond formation of electrophoretically transferred 4S RNA. Segments of composite gels containing $^{32}$P-labeled 4S RNA were incubated in phosphate buffer and transferred to DBM paper at 10V/cm. DBM-papers carrying RNA were covered with Saran Wrap and incubated for times varying from 0 to 30 hours at 20°C (○) or -80°C (●). After incubation each DBM-paper carrying RNA was washed separately with 4 changes of 2x SSC, 0.1% SDS at 65°C for 24 hours. The washes were counted to assess th amount of RNA non-covalently bound by DBM-paper. The RNA bound to the DBM-papers after washing was hydrolyzed by incubating the DBM-paper carrying RNA in 1N NaOH overnight at 65°C. RNA coupling efficiencies were determined as described in TABLE I.

molecules to DBM-paper involves a two-step reaction sequence.

Time-dependent decay of ability to form covalent bonds

The decay rate of reactive diazonium groups on DBM-paper often exceeds the rate at which macromolecules can be transferred from gels to DBM-paper (3-6). The net effect of such rapid decay is a low covalent coupling efficiency. The decay rate of covalent coupling efficiency under our conditions was determined by electrophoresing 4S RNA from gel segments onto DBM-paper at various times after preparation of the paper. The DBM-paper was kept in 25 mM phosphate buffer at 4°C until used. RNA coupling to DBM-paper was slightly more efficient at 1 and 2 hours after preparation than when used immediately, as shown in Figure 4. We have observed this phenomenon repeatedly and suspect that prolonged washing of DBM-paper removes inhibitors of covalent coupling generated during the process of diazotization.
The covalent coupling efficiency of DBM-paper for 4S RNA began to decline rapidly 3-4 hours after diazotization, with approximately 50% of the initial coupling activity lost during the first 6-8 hours and less than 2% remaining after 24 hours.

pH optimum for covalent coupling of RNA

Alwine et al.(2) indicated that coupling of nucleic acids to DBM-paper was more efficient at pH 6.5 than at pH 8.0. The optimum pH for electrophoretic transfers of macromolecules to DBM-paper was assessed by electrophoresing 4S RNA onto DBM-paper preincubated at 4°C for various periods of time in buffers rang-

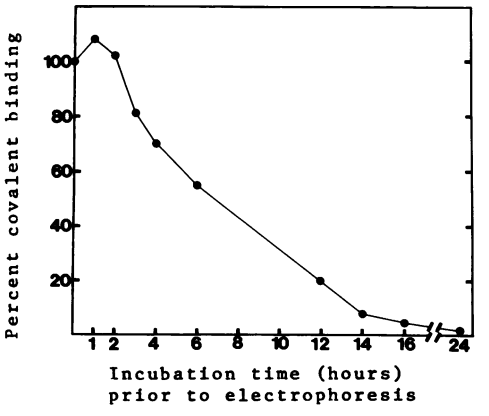


Figure 4. Time-dependent decay of covalent bond forming ability.Labeled RNA,as used in Figure 3, was electrophoretically transferred to DBM-paper at varying times after preparation of the paper. The papers were incubated overnight to allow for maximum covalent bond formation, and then washed and counted as in Figure 3. The control (100%) was covalent coupling efficiency of freshly prepared DBM-paper.

ing in pH from 4.0 to 9.0. Figure 5 shows that the covalent coupling efficiency of 4S RNA to DBM-paper was markedly influenced by pH. Preincubation of DBM-paper at pH 9 resulted in the most rapid decline in covalent coupling activity, while preincubation at pH 4 and 5 were most effective in prolonging the covalent coupling activity of DBM-paper. These results confirm and extend those obtained by Alwine et al.(2).

Non-covalent binding of RNA and DNA to DBM-paper

After prolonged incubation,DBM-paper lost the ability to form covalent bonds with macromolecules (as shown in Figure 4) but it did retain a capacity for non-covalent, presumably electrostatic binding of nucleic acids. DBM-paper incubated in 50mM sodium phosphate,pH 5.5, at 4°C for 24-30 hours bound electrophoretically transferred DNA and RNA with a capacity of approximately 25-30µg/ $cm^2$. This was about the same capacity as freshly prepared DBM-paper (2). However the DNA and RNA bound in this manner were quantitatively removed by overnight incubation of DBM-paper in 2x SSC, 0.1% SDS.

We utilized the non-covalent binding of RNA to DBM-paper for isolation of specific RNAs from gels. For example N. crassa tRNAs, labeled with $^{32}P$ and separated by two-dimensional gel electrophoresis, were electrophoretically transferred to DBM-paper which had lost all capacity for covalent coupling. The DBM-paper was autoradiographed and spots corresponding to four tRNAs were cut

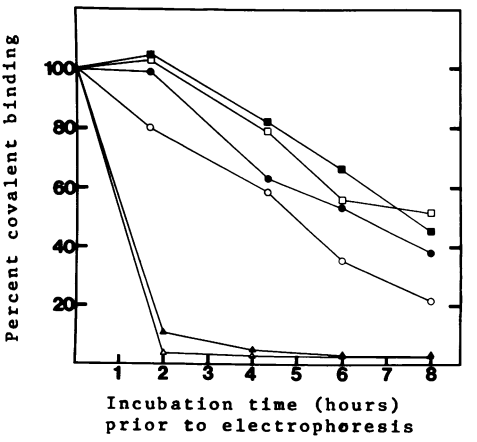


Figure 5. Optimum pH for stabilization of covalent coupling of RNA by DBM-paper. The procedure was identical to that of Figure 4 except that the DBM-paper was preincubated in six different buffers ranging in pH from 4 to 9. Buffers were as follows: 50mM sodium acetate/acetic acid,pH 4 (■), and pH 5 (□); 50mM sodium phosphate, pH 6 (●), and pH 7 (○); 50mM sodium borate/boric acid,pH 8(▲), and pH 9 (△).

out, eluted in 2x SSC, 0.1% SDS, re-electrophoresed on a second two-dimensional gel and again transferred to DBM-paper. The positions of the four spots in the second gel correspond exactly to their positions in the original gel as shown in Figure 6.

In a second experiment we studied a much larger RNA, the 25S precursor of p23S rRNA in E.coli mutant AB105. This RNA, which is found on polyribosomes (A.D.,unpublished observation) was separated in a composite gel along with numerous other precursor rRNAs from this mutant strain deficient in the rRNA processing enzyme RNase III (Figure 7, slot 1).After transfer of the $^{32}$P-labeled RNA to DBM-paper which had lost covalent coupling capacity, the 25S RNA was eluted in 25mM Tris-HCl, 10mM $MgCl_2$, 300mM $NH_4Cl$, pH 7.6 buffer overnight at 4°C. The RNA was concentrated and dialyzed to reduce $NH_4Cl$ to 130mM. An aliquot was treated with RNase III and electrophoresed into a composite gel adjacent to an untreated sample. The conversion of 25S to p23S rRNA, as shown in Figure 7, slots 2 and 3, indicated that electrophoretic transfer and elution of RNA from DBM-paper yielded RNAs susceptible to enzymatic hydrolysis and unaltered in electrophoretic mobility.

Non-covalent binding of polyribosomes to DBM-paper

E.coli polyribosomes, labeled in vitro with $^{32}$pCp (11) and electrophoretically separated in a composite gel (14), were transferred to DBM-paper incapable of covalent bond formation using a buffer containing 2mM $MgCl_2$, 50mM MES (2(N-Morpholino) ethane sulfonic acid), pH 7.0. Electrophoretic conditions were as described above for transfer of large RNAs. Monosomes and disomes were eluted from the paper in 0.3 ml of 2x SSC, 0.1%SDS overnight at 4°C. The RNAs in each sample, separated from the

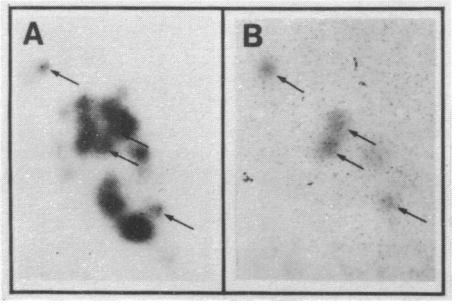


Figure 6. Isolation and re-electrophoresis of N.crassa tRNA non-covalently bound to DBM-paper. Panel A: Autoradiograph of $^{32}$P-labeled tRNA after electrophoretic transfer from gel onto DBM-paper incapable of covalent bond formation. Panel B: Autoradiograph of four tRNAs eluted from DBM-paper of panel A, re-electrophoresed in a two-dimensional gel and,again, transferred to DBM-paper.

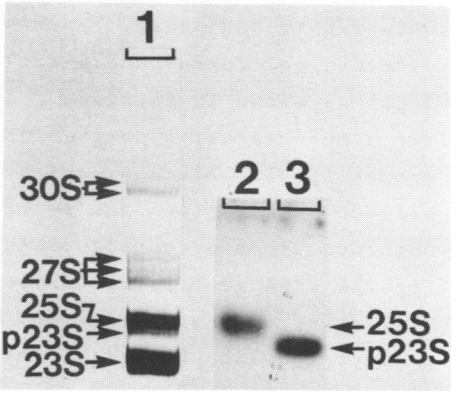


Figure 7. RNase III treatment of precursor rRNA isolated from DBM-paper. $^{32}$P-labeled RNA of strain AB105 separated in a composite gel and stained with Stains-All(14)(slot 1). Autoradiograph of 25S RNA eluted from DBM-paper and re-electrophoresed into a tighter gel (3.5% acrylamide,0.5% agarose) (slot 2), and same sample after RNase III treatment (slot 3).

protein by SDS, were electrophoresed directly into a composite gel and the gel autoradiographed. Figure 8 shows a stained gel pattern of polyribosomes (slot 1) and an autoradiograph of the labeled polyribosomes on DBM-paper (slot 2). The intact nature of the rRNAs eluted from the monosome and disome regions of the DBM-paper is seen in slots 3 and 4.

A simultaneous transfer and separation of the ribosomal protein and rRNA can be achieved by soaking the gel briefly in 8M urea prior to electrophoretic transfer. Urea dissociates the protein from the rRNA and the two components migrate in opposite directions onto DBM-paper placed on either side of the gel.

Covalent binding of polyribosomes and ribosomal precursor

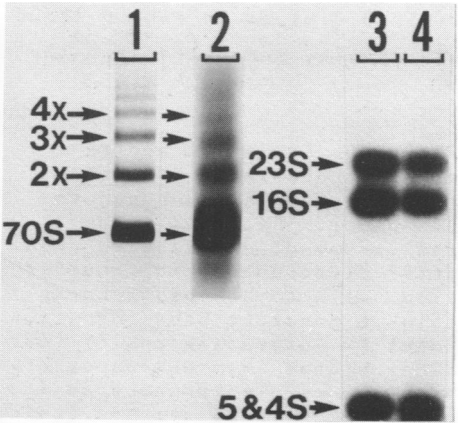


Figure 8. Non-covalent binding of polyribosomes to DBM-paper. $^{32}$P-labeled bacterial polyribosomes were electrophoretically separated in a composite gel and stained (14)(slot 1), or transferred to DBM-paper and autoradiographed (slot 2). RNAs recovered from monosome and disome regions of DBM-paper were electrophoresed in a composite gel and autoradiographed (slots 3 and 4). 2x,3x and 4x represent polyribosomes of disome, trisome and tetrasome size.

particles can also be achieved using freshly prepared DBM-paper and conditions as described above (data not shown).

Hybridization of $^{32}$P-labeled RNA to DBM-paper carrying DNA

The ability of electrophoretically transferred DNA, covalently bound to DBM-paper, to hybridize with $^{32}$P-labeled RNA probes was tested using plasmid DNA obtained from pMF2, a hybrid plasmid containing the complement of sequences homologous to the 26S, 17S and 5.8S rRNAs from N.crassa (16). pMF2 was digested with restriction endonucleases BAM H1 and EcoR1 and the fragments were separated by electrophoresis in an agarose gel as shown in Figure 9, slots 1 and 2. After electrophoretic transfer to freshly prepared DBM-paper they were hybridized with RNA probes. As shown in Figure 9, $^{32}$P-labeled 26S and 17S rRNA hybridized to appropriate restriction fragments of pMF2 DNA (16). Background radioactivity resulting from non-specific binding of RNA to DBM-paper was comparable to or lower than that obtained with nitrocellulose (unpublished observations).

Hybridization of $^{32}$P-labeled DNA to DBM-paper carrying RNA

Total cellular RNA from N.crassa was electrophoresed into a composite gel and then electrophoretically transferred onto DBM-paper at 10V/cm for 6 hours in phosphate buffer. The paper was pretreated as described in Methods and then hybridized with $^{32}$P-labeled pMF2 DNA probe, prepared by nick-translation (20). Hybridizations were performed as described by Alwine et al.(2).

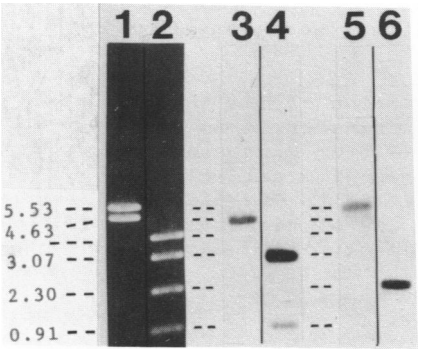


Figure 9. Hybridization of RNA to DNA restriction fragments electrophoretically transferred to DBM-paper. pMF2 DNA digested with restriction endonucleases BAM H1 (slot 1) and EcoR1 (slot 2) was electrophoretically separated on a 1% agarose gel and stained with ethidium bromide. Gel strips identical to 1/2 were electrophoretically transferred to DBM-papers,3/4 and 5/6, and then hybridized as described in Methods with $3x10^4$ cpm of 26S rRNA probe (slots 3 and 4) or $2.1x10^4$ cpm of 17S rRNA (slots 5 and 6) ($3x10^3$ cpm/µg and about $10^4$ cpm/ml).

The autoradiograph (Figure 10) shows bands corresponding to the three species of N.crassa rRNA. In addition weak but unequivocal hybridization was also obtained for at least two bands in the region between 17S and 5.8S rRNA. Kim et al.(26) have reported the presence of three small stable RNAs isolated from long term $^{32}$P-labeled N.crassa ranging in size from larger than 7S to smaller than 17S. If these RNAs were ribosomal in origin they could be responsible for the weak hybridization observed with pMF2 probe. The identity of these RNA species remains to be established, however.

Electrophoretic transfer of protein to DBM-paper

E.coli 70S ribosomal proteins, labeled with $^{14}$C-leucine, were separated by gel electrophoresis (22) and stained with Coomassie Blue (Figure 11,slot 1). A duplicate gel (unstained) was electrophoretically transferred to DBM-paper and the DBM-paper autoradiographed. The pattern obtained is shown in Figure 11, slot 2. Proteins ranging in size from 6,000 to 65,000 daltons were electrophoretically transferred to DBM-paper from a 10% acrylamide-urea gel in 2 hours at about 10V/cm. The gel from which the proteins were transferred showed no detectable bands upon autoradiography indicating that there was essentially complete transfer.

Similar results were obtained when ribosomal proteins were transferred from a two-dimensional urea gel (23) (data not shown). Transfer of proteins from an SDS gel (24) was also successful (data not shown) but required modifications in the procedure as described in Methods. It was necessary to keep

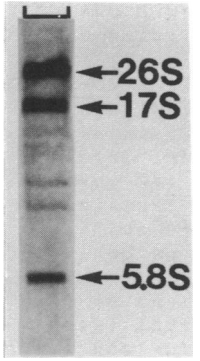


Figure 10. Hybridization of DNA to rRNA electrophoretically transferred to DBM-paper. Total cell RNA from N.crassa was fractionated on a composite gel, electrophoretically transferred to DBM-paper and hybridized with $10^6$ cpm of nick-translated pMF2 DNA (1-3x$10^6$ cpm/$\mu$g, 5x$10^5$ cpm/ml).

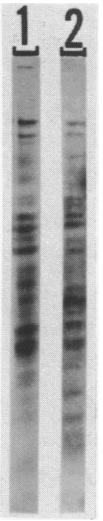


Figure 11. Electrophoretic transfer of ribosomal proteins to DBM-paper. Slot 1: $^{14}C$- leucine labeled 70S ribosomal proteins electrophoretically separated in a 10% acrylamide gel and stained. Slot 2: Autoradiograph of DBM-paper after electrophoretic transfer of an identical, unstained gel strip.

SDS in the soak buffer while removing Tris and glycine to avoid precipitation of the proteins in the gel. However SDS was omitted from the reservoir buffer during electrophoretic transfer to DBM-paper to prevent the SDS-protein complexes from passing right through the DBM-paper.

## DISCUSSION

Here we describe an electrophoretic technique for the transfer of DNA, RNA, protein and ribonucleoprotein particles onto DBM-paper. This method of transfer has several advantages over the conventional method of blotting. All sizes of macromolecules, including very large DNA, RNA and polyribosomes, are transferred to the DBM-paper. The process is rapid, thus achieving complete transfer within the time the paper is capable of forming covalent bonds, even when transferring from a gel composed of 20% acrylamide. Transfer is direct, without any lateral diffusion, thus preserving the sharpness of the original gel pattern on the DBM-paper, and permitting the detection of very small amounts of DNA and RNA. The molecules are transferred intact allowing for binding of large RNAs and single and double stranded DNAs to the DBM-paper without the chemical treatment necessary for transfer by the blotting procedure (2,4). The DNA

and RNA thus transferred remain competent for hybridization to specific radioactive probes. The efficiency of transfer of intact RNA (up to 80%) exceeds that achieved by blotting to DBM-paper following alkali hydrolysis (2) and DNA transfer efficiency (greater than 80%) is equal to that recently described by Wahl et al.(4). Together these factors provide a much improved transfer procedure over blotting with an almost quantitative covalent coupling of intact transferred material.

Electrophoretic transfer of protein from urea-containing gels is about 5 to 10 times more efficient than that reported by the blotting method (5). A less than quantitative transfer is achieved, however, from SDS gels although the original gel pattern is preserved. This was also observed by Towbin et al. (27) with electrophoretic transfer of proteins to nitrocellulose. Proteins electrophoretically transferred to DBM-paper from both urea and SDS gels successfully bind antibody in the manner already demonstrated for blot-transferred proteins (5) (E.Stellwag & D. Winkelmann and J. Beachy & D. Goldman, unpublished observations).

The speed and efficiency of the electrophoretic transfer method has permitted us to examine and confirm some of the parameters reported for the covalent bond formation on DBM-paper (2). In particular we observe that the covalent bond is formed within 2.5 hours after transfer. The DBM-paper's ability to form the covalent bond remains high for the first 2 to 4 hours after preparation if kept at pH 4-6 and 4 C, but then declines dramatically over 24 hours. The marked decrease in covalent coupling efficiency after prolonged incubation accounts for the reduced coupling efficiency associated with transfer by blotting (2-6), and indicates that material transferred after 10-16 hours couples very poorly. Since complete electrophoretic transfer of intact DNA, RNA and protein from gels to DBM-paper can generally be accomplished in 2-6 hours, a coupling efficiency of at least 60-80% can be expected for a wide variety of transfers.

Several different buffer systems may be successfully employed for the electrophoretic transfer of macromolecules to DBM-paper. The sodium phosphate buffer described here was quite satisfactory for most transfers although acetate buffer, when

used in two-dimensional protein gel electrophoretic separations, can then be more conveniently used for subsequent transfer to DBM-paper. Optimum transfer may be achieved with a zwitterionic buffer such as MES or MOPS at a pH equal to its isoelectric point, but these buffers are quite expensive. A home-made electrophoretic transfer apparatus with two electrode wires in a tray is acceptable for transfer of some materials but a considerably more uniform, rapid and reproducible transfer is achieved with electrophoretic destaining plates as described in Methods and Materials.

The binding of macromolecules to DBM-paper incapable of covalent bond formation provides a new and useful preparative method which has not been achieved using conventional blotting procedures. Particles of all sizes and in all types of gels can be electrophoretically transferred to the DBM-paper and then eluted in high salt (0.3M). RNase III cleavage of E.coli 25S precursor rRNA, isolated from DBM-paper, demonstrates that the RNA remains a suitable substrate for the enzyme and is unaffected by this preparative method. A rapid, simultaneous isolation of both protein and RNA from ribosomes in a composite gel is achieved by the bidirectional electrophoretic transfer onto two papers on either side of the gel. By washing the DBM-paper in 1M NaCl to remove all non-covalently bound material, and re-equilibration in low salt buffer, the paper may be reused repeatedly.

Noyes and Stark (28) previously indicated that single strandedness was a requirement for covalent coupling of nucleic acids to DBM-paper. In assessing the transfer and coupling efficiency of DNA restriction fragments we found that non-denatured EcoR1 restriction fragments of pMF2, labeled with $^{32}P$ using terminal deoxynucleotidyl transferase (21),were covalently coupled to DBM-paper with excellent efficiency (77% - see Table I). Although these results seemed to contradict the published observations we observed only 10% binding of EcoR1 fragments labeled at the staggered cut ends with $^{32}P$ by polynucleotide kinase, and 1-2% binding of SmaI generated fragments with blunt ends. We suggest that the terminal adenosine labeled EcoR1 restriction fragments ( average of 6 adenosines/3' OH EcoR1 generated end)

are coupling to DBM-paper by virtue of their single stranded tails. Indeed we found that $^{32}$P AMP, electrophoretically transferred to DBM-paper, bound covalently with a relative affinity of approximately 40-45% of that of GMP. CMP and UMP affinities were approximately 20% and 2-4% respectively (data not shown). The efficient coupling to DBM-paper of DNA which is largely double stranded may be useful in the localization of DNA-binding proteins to particular DNA restriction fragments carrying sequences recognized by these proteins.

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