Proc. Natl. Acad. Sci. USA
Vol. 76, No. 8, pp. 3683–3687, August 1979
Biochemistry

Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulfate

(Southern transfers/two-phase hybridizations/partial depurination/nick-translated probes/recombinant DNA)

GEOFFREY M. WAHL, MICHAEL STERN, AND GEORGE R. STARK

Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

Communicated by I. Robert Lehman, May 7, 1979

ABSTRACT **We describe a technique for transferring electrophoretically separated bands of double-stranded DNA from agarose gels to diazobenzyloxymethyl-paper. Controlled cleavage of the DNA *in situ* by sequential treatment with dilute acid, which causes partial depurination, and dilute alkali, which causes cleavage and separation of the strands, allows the DNA to leave the gel rapidly and completely, with an efficiency independent of its size. Covalent attachment of DNA to paper prevents losses during subsequent hybridization and washing steps and allows a single paper to be reused many times. Ten percent dextran sulfate, originally found to accelerate DNA hybridization in solution by about 10-fold [J. G. Wetmur (1975) *Biopolymers* 14, 2517–2524], accelerates the rate of hybridization of randomly cleaved double-stranded DNA probes to immobilized nucleic acids by as much as 100-fold, without increasing the background significantly.**

Southern's procedure (1) for transferring restriction fragments from agarose gels to nitrocellulose has been an essential part of recent advances in analyzing and purifying many DNA fragments and has also been used more recently to identify mutant globin genes in individuals with thalessemia (2) or sickle cell trait (3). However, several aspects of this method have not yet been optimized: (*i*) Large restriction fragments are not transferred from agarose gels efficiently, and fragments smaller than 0.3–0.5 kilobase (kb) do not bind well to nitrocellulose. (*ii*) Some of the DNA bound noncovalently to nitrocellulose is removed by stringent posthybridization washes, reducing the intensity of the signal and limiting repeated uses of the transfer. (*iii*) The technique is relatively slow; up to 2 weeks can be required to detect fragments derived from unique genes of higher eukaryotes.

One modification of Southern's technique follows from our previous work, which showed that RNA and single-stranded DNA could be linked covalently to diazobenzyloxymethyl (DBM)-cellulose and used in hybridization reactions (4), that RNA could be transferred from agarose gels to DBM-paper (5), and that small fragments of DNA could be transferred from composite agarose–acrylamide gels to DBM-paper (6). Transfer of DNA fragments of any size from agarose gels to paper has now been optimized. An important improvement follows from Wetmur's finding (7) that 10% dextran sulfate greatly accelerates hybridization reactions in solution. This anionic polymer gives an even larger acceleration in a two-phase system with double-stranded, randomly cleaved, denatured DNA as probe.

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.

MATERIALS AND METHODS

General Methods. Conditions for cleaving the DNA samples with restriction enzymes, for separating the fragments according to size by electrophoresis in agarose gels (8), and for preparing nick-translated probes (9) are given by Wahl *et al.* (10).

Determination of DNA Fragment Lengths after Partial Depurination and Strand Cleavage in Agarose Gels. DNA samples, separated by electrophoresis through a 0.8% agarose gel until the bromcresol purple dye marker was 1 cm from the origin, were depurinated partially and cleaved by sequential treatment with acid and alkali as described below. The gel was equilibrated with 30 mM NaOH/2 mM EDTA (eight changes for 15 min each), and electrophoresis was resumed until the dye marker was approximately 6 cm from the origin (16 hr). A sample of λ DNA from strain $J^-_{am}Z^-_{zm}$Vir (11), digested with restriction endonuclease *Hin*dIII and not depurinated, provided single-stranded molecular weight markers. Fragments were visualized with 254-nm light after equilibrating the gel with 0.2 M sodium phosphate (pH 6.5) containing 1 μg of ethidium bromide per ml.

Preparation of End-Labeled λ DNA Fragments. λ DNA (10 μg) from $J^-_{am}Z^-_{am}$Vir was cleaved with 10 units of *Hin*dIII for 1 hr at 37°C in a buffer containing 20 mM Tris·HCl (pH 7.4), 60 mM NaCl, 7 mM $MgCl_2$, 100 μg of bovine serum albumin (Bethesda Research Laboratories, Rockville, MD) per ml, and 2 mM dithiothreitol, in a total volume of 60 μl. Addition of [α-^{32}P]dCTP and [α-^{32}P]dGTP to the staggered ends of the fragments was catalyzed by avian myeloblastosis virus reverse transcriptase. The *Hin*dIII digest was diluted with an equal volume of 20 mM Tris·HCl, pH 7.4/20 mM NaCl/400 μM dATP/400 μM dTTP/50 μCi each of the ^{32}P-labeled triphosphates (Amersham, 300 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels). Sixteen units of reverse transcriptase (from Joseph Beard, Life Sciences, St. Petersburg, FL) was added, the mixture was incubated at 37°C for 1.5 hr, and the reaction was stopped by adding 0.1 vol of 1% Sarkosyl/125 mM EDTA, followed by heating to 70°C for 5 min. Free nucleotides were removed by filtration through a column of Bio-Gel P-60, equilibrated with 10 mM Tris·HCl, pH 7.4/1 mM EDTA.

Preparation of End-Labeled φX174 Viral DNA. The DNA (5 μg, kindly provided by Y. Shlomai, Biochemistry Department, Stanford University) was incubated at room temperature with 0.20 M HCl for 5 min, followed by 0.50 M NaOH for 30

Abbreviations: kb, kilobases; DBM, diazobenzyloxymethyl; NaCl/Cit, 0.15 M NaCl/15 mM trisodium citrate.

min, yielding fragments 100–1000 bases long. The DNA was precipitated with ethanol and dissolved in 200 μl of 20 mM Tris·HCl, pH 8.7/1 mM $MgCl_2$. The 5′-phosphoryl groups were removed by incubation for 3 hr at 37°C with calf intestine alkaline phosphatase (kindly provided by D. Goldberg, Biology Department, California Institute of Technology). After phenol extraction and precipitation with ethanol, the 5′ termini of the fragments were labeled (12) with [γ-^{32}P]ATP (3000 Ci/mmol, Amersham) by using T4 polynucleotide kinase (PL Biochemicals).

PROCEDURE

DBM-Paper. Prepare DBM-paper according to Alwine *et al.* (5) with the following modifications: Wash the paper with water and then acetone (not benzene) after the 135°C step. Use 1 M sodium acetate buffer (pH 4.0) instead of 20 mM sodium phosphate buffer (pH 6.5) as a wash just after diazotization. Lower pH increases both the stability of diazonium groups and the amount of nucleic acid bound.

Transfer of DNA from Agarose Gels to DBM-Paper or Nitrocellulose. The protocol is designed for a 150-ml agarose gel (13.5 × 14.5 × 0.8 cm). All procedures are at room temperature. Bromcresol purple, the dye marker used for electrophoresis, is also a convenient indicator of pH changes in the rest of the procedure. The concentration of HCl used to obtain fragments of 1–2 kb should be determined experimentally for gels of thickness other than 0.8 cm. Transfer is equally efficient from gels with agarose concentrations between 0.3 and 1.0%. We have not tested buffers other than Tris acetate, but we expect that they should give equivalent results.

Place the gel in a pan containing 250 ml of 0.25 M HCl, shake it gently for 15 min, decant the acid, and repeat. Wash the gel briefly with distilled water to remove residual acid, and then wash the gel with two 250-ml portions of 0.5 M NaOH/1 M NaCl for 15 min each. For transfer to DBM-paper, decant the NaOH/NaCl solution, wash the gel briefly with water, and shake it with two 250-ml portions of 1 M sodium acetate buffer (pH 4.0) (47.6 ml of glacial acetic acid plus 14.8 g of anhydrous sodium acetate per liter) for 30 min each. Prepare the DBM-paper just before transfer. For transfer to nitrocellulose, neutralize the gel with 0.5 M Tris·HCl, pH 7.4/3 M NaCl (1).

Transfer of partially cleaved DNA fragments from agarose gels to solid supports is complete after only 2 hr with the following simplified procedure: Place the gel on top of two 20 × 30 cm sheets of Whatman 3MM paper saturated with 1 M sodium acetate buffer, pH 4.0 [use 3 M NaCl/300 mM trisodium citrate (20 times concentrated NaCl/Cit) for transfer to nitrocellulose]. Place Saran wrap on the Whatman paper around the gel to prevent contact between the dry paper to be placed above the gel and the wet paper beneath. Position the DBM-paper or nitrocellulose on top of the gel. Remove any air bubbles from regions where the gel and paper are in contact. Add two sheets of dry Whatman 3MM paper, then a 3-inch layer of paper towels, and finally a light weight, to ensure even contact. Transfer fragments for 2 hr or longer, and do not add buffer to the saturated paper during transfer.

Pretreatment, Hybridization, and Detection of Specific DNA Sequences. Hybridization with dextran sulfate can give high backgrounds. Nick-translated probes with specific activity >5 × 10^7 cpm/μg and a 0.5- to 1-kb single strand size give the best results. Sporadic high backgrounds are minimized by following the procedure below exactly.

Place the paper (9 × 13.5 cm) in 10 ml of 50% formamide (99%, A.C.S. grade, Matheson, Coleman & Bell)/5 times concentrated NaCl/Cit/5 times concentrated Denhardt's (13) reagent [Denhardt's reagent contains 0.02% (wt/vol) each of bovine serum albumin, polyvinyl pyrrolidone, and Ficoll (M_r 400,000)]/50 mM sodium phosphate, pH 6.5/1% glycine/250–500 μg of sonicated, denatured salmon sperm DNA (Sigma) per ml in a polyethylene bag. Incubate at 42°C for at least 1 hr. Draw a rod over the opened bag to extrude as much liquid as possible, but do not blot the paper. Prepare 10 ml of a solution of 50% formamide, 5 times concentrated NaCl/Cit, Denhardt's reagent, 20 mM sodium phosphate (pH 6.5), 100 μg of sonicated, denatured salmon sperm DNA per ml, and 10% sodium dextran sulfate 500 (Pharmacia). The dextran sulfate is added as a 50% (wt/vol) aqueous solution, which is slightly yellow and quite viscous. Add 9 ml of this mixture to the bag, wetting the paper thoroughly. Heat the remaining 1 ml to 65°C for a few minutes to facilitate subsequent mixing with the probe. Denature the probe at 95°C in 10 mM Tris·HCl, pH 7.4/1 mM EDTA for 5 min, and cool in an ethanol/ice bath. Add the probe to the heated hybridization buffer, mix vigorously, and add the mixture to the bag. Seal the bag near the paper without trapping air bubbles and mix the solution thoroughly. Incubate the bag at 42°C for 4–16 hr, depending on the source and amount of DNA and the quantity of probe. This procedure may also be used for hybridizing probes to RNA-paper (unpublished results). In this case, 0.1% sodium dodecyl sulfate should be included during prehybridization and hybridization to inhibit ribonuclease. Sodium dodecyl sulfate is not needed with DNA-paper.

Wash the DNA-paper with three 250-ml portions of 2 times concentrated NaCl/Cit/0.1% sodium dodecyl sulfate for 5 min each at room temperature and then with two 250-ml portions of 0.1 concentrated NaCl/Cit/0.1% sodium dodecyl sulfate at 40°–50°C for a total of 30 min (14). The background detected with a monitor should be very low. If it is unacceptably high, continue washing with this buffer for an additional 30 min. Expose the x-ray film to the paper at −70°C by using a Du Pont Lightning Plus intensifying screen.

RESULTS AND DISCUSSION

Cleavage of DNA in the Gel by Partial Depurination. Large fragments of DNA are not transferred well in Southern's original procedure (1) but can be transferred with high efficiency if they are cleaved partially in the gel by using 254-nm light in the presence of ethidium bromide (15). However, progressive solarization of the ultraviolet filters causes transfer efficiency to decrease with increasing use.

DNA can be depurinated partially with acid and then cleaved at the depurination sites with alkali (16). Alkali also denatures DNA to single strands, which are required for binding to DBM-paper (4) or nitrocellulose (17). To determine the sizes of fragments obtained under different conditions, intact DNA from bacteriophage λ (strain $J^-_{am}Z^-_{am}$Vir, 50 kb), λ DNA digested with *Hin*dIII (0.56–22.7 kb), and hamster DNA digested with *Pvu* II were run a short distance into an agarose gel, cleaved partially *in situ* by sequential treatment with HCl and NaOH, and then separated by electrophoresis in alkali to determine the lengths of the single-stranded fragments (Fig. 1). λ DNA digested with *Hin*dIII but not cleaved with acid and alkali was run in parallel to provide size markers. All the cleaved fragments were 1- to 2-kb long, appreciably longer than the minimum size required for forming stable hybrids under the conditions used (18).

Transfer of DNA to Paper. Restriction fragments obtained by cleaving λ $J^-_{am}Z^-_{am}$Vir DNA (11) with *Hin*dIII were labeled at their 3′ termini with α-^{32}P-labeled deoxynucleoside triphosphates by using reverse transcriptase, fractionated according to size by electrophoresis (8), depurinated and cleaved *in situ*, and transferred to DBM-paper or nitrocellulose. As

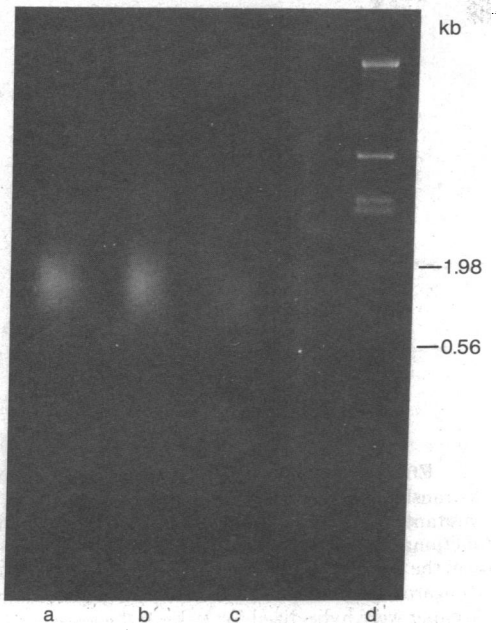

FIG. 1. Fragments resulting from partial depurination and alkaline cleavage of DNA in agarose gels. The samples were run 1 cm into a 0.8% agarose gel, depurinated partially, and cleaved. The gel was then equilibrated with 30 mM NaOH/2 mM EDTA, and electrophoresis was resumed under denaturing conditions. A separate lane containing λ DNA digested with *Hin*dIII was denatured *in situ* to provide size standards. After electrophoresis the gel was equilibrated with 0.2 M sodium phosphate, pH 6.5/1 μg of ethidium bromide per ml, and the DNA fragments were visualized with 254-nm light. Lanes: a, intact λ DNA; b, λ DNA digested with *Hin*dIII; c, hamster DNA digested with *Pvu*II; d, λ size standard.

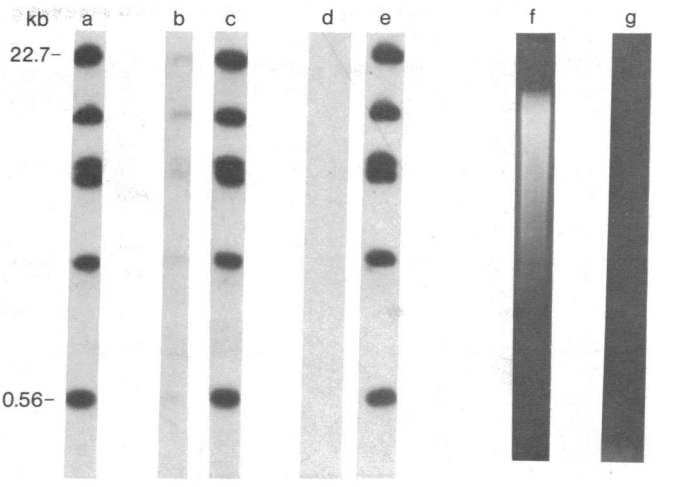

FIG. 2. Transfer of partially depurinated restriction fragments from agarose gels to DBM-paper or nitrocellulose. Bacteriophage λ $J^-_{am}Z^-_{am}$Vir DNA was cleaved with endonuclease *Hin*dIII, and the staggered ends were filled in with ^{32}P-labeled nucleotides by using reverse transcriptase. The labeled DNA fragments were then fractionated on a 0.7% agarose gel, depurinated partially with 0.25 M HCl, and processed further as described in *Procedure*. The autoradiograms are: a, gel dried immediately after treatment; b, gel after transfer to DBM-paper; c, paper after transfer from gel shown in b; d, gel after transfer to nitrocellulose; e, nitrocellulose after transfer from gel shown in d. Tracks f and g show the ethidium bromide staining patterns of hamster DNA digested with *Pvu* II before and after transfer.

shown in Fig. 2, transfer from a 0.7% agarose gel was complete in 2 hr, and fragments in the range 0.56 to 22.7 kb were transferred with equally high efficiency to either solid support. Reiser *et al.* (6) showed that DNA fragments much smaller than 0.56 kb can be transferred to DBM-paper (but not to nitrocellulose) and detected with DNA probes. Restriction fragments from hamster DNA were transferred efficiently from the gel to DBM-paper in 2 hr (compare tracks f and g of Fig. 2). Undigested hamster DNA and oligomers of phage λ are also transferred well (data not shown); the efficiency of transfer seems to be completely independent of the size of the DNA.

Approximately 80% of the labeled DNA present in the gel before transfer remains bound to DBM-paper after washing with alkali (0.4 M NaOH for 30 min at room temperature) and is not removed by further washes. Because little or no DNA is lost from the gel during pretreatment with acid and alkali, about 20% is not bound stably to DBM-paper. Stark and Williams (19) found that a similar fraction of DNA binds to DBM-paper in 80% dimethyl sulfoxide.

To estimate the amount of DNA that can be transferred stably to DBM-paper, trace amounts of ^{32}P-labeled *Hin*dIII restriction fragments of λ $J^-_{am}Z^-_{am}$Vir were fractionated by electrophoresis in the presence or absence of 10–15 μg of hamster DNA digested with *Eco*RI. About 80% of the labeled DNA was bound stably to the paper in each case. In another experiment, trace amounts of ^{32}P-labeled *Hin*dIII fragments from λ $J^-_{am}Z^-_{am}$Vir were fractionated by electrophoresis in the presence of increasing amounts of identical but unlabeled restriction fragments. The efficiency of transfer was the same in each experiment, even when the amount of DNA exceeded the capacity of the gel (about 2 μg per 6 × 8 × 2 mm well).

Hybridization of Labeled Probes to DNA-Paper. Hybridization of DNA-paper with nick-translated probes (9) was tested by using DNA from mutant hamster cells that have multiple copies of the gene encoding a multifunctional protein that catalyzes the first three steps of UMP synthesis (10). The probe was prepared from a hybrid plasmid containing a 2.3-kb insert complementary to the 3′-proximal region of the mRNA for this protein (10). DNA isolated from wild-type and mutant cells was cleaved with endonuclease *Eco*RI, fractionated on an agarose gel, transferred to DBM-paper, and hybridized with the probe. As shown in Fig. 3 *left*, the major fragment is 19-kb long, and the amount of hybridization closely reflects the number of genes in each cell line. When similar digests with *Pvu* II were analyzed (Fig. 3 *right*), the probe hybridized with five fragments which range from 0.7 to 1.6 kb (lanes b–d). Hybridization with a *Pvu* II digest of the hybrid plasmid ($pCAD_{41}$) resulted in a very different pattern (Fig. 3 *right*, lane a), in which the smallest fragment was 0.39 kb. Such major differences in restriction patterns between genomic DNA and a cloned cDNA indicate that sequences in the gene and mRNA are not contiguous (10).

Fig. 3 shows that both small and large restriction fragments can be detected and that one can detect fragments derived from a unique gene of a higher eukaryote in about 1 day by using less than 5 μg of genomic DNA. Furthermore, the extent of hybridization to a specific sequence reflects the number of copies of that sequence in the genome. Because the transfer is virtually quantitative and independent of size, it should be possible to distinguish differences in gene copy number as small as 2-fold in parallel comparative experiments.

Although restriction fragments are transferred to nitrocellulose and DBM-paper with equal efficiency (see Fig. 2), the extent of hybridization to DNA-paper is usually 2–3 times greater than to DNA-nitrocellulose after the stringent washes (0.1 concentrated NaCl/Cit/0.1% sodium dodecyl sulfate at 50°C for 1–2 hr) often required to give low backgrounds, reflecting loss of DNA from the nitrocellulose (data not shown). Washing DNA-paper with 0.4 M NaOH at 37°C for 30 min

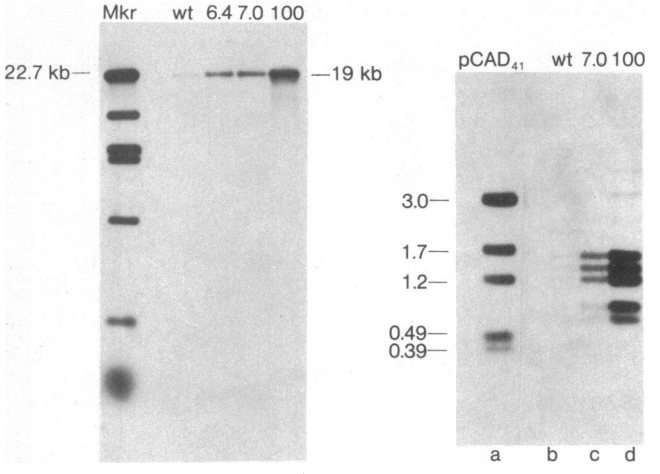

FIG. 3. Detection of restriction fragments on DNA-paper. DNA from mutant (25–5 μg) or wild type (5–10 μg) hamster cells was digested with either nuclease *Eco*RI (*Left*) or *Pvu* II (*Right*). The fragments were fractionated according to size on 0.5% (*Eco*RI digest) or 1% (*Pvu* II digest) agarose gels and transferred to DBM-paper. The DNA-papers were treated for 1 hr with hybridization buffer plus 1% glycine but without dextran sulfate or probe and then hybridized for 12 hr in hybridization buffer containing 10% dextran sulfate and 2×10^6 cpm of nick-translated probe (2×10^5 cpm/ml, 3×10^7 cpm/μg). The washed papers were autoradiographed for 12 hr with DuPont Kronex film (*Left*) or for 18 hr with Kodak XR-5 film (*Right*) and a Kodak Lightning Plus intensifying screen at −70°C. The level of multifunctional protein in the mutant cells relative to the level in the wild-type cells (wt) is indicated above each lane. Mkr, *Hin*dIII-digested λ $J^-_{am}Z^-_{am}$ Vir DNA.

removes all the hybridized probe but little or none of the covalently bound unlabeled DNA, and rehybridization with the same probe yields signals equivalent to those obtained initially. A single DNA-paper can be used many times with the same or with different probes.

Dextran Sulfate Accelerates Two-Phase Hybridizations. Wetmur (7) observed that anionic dextran polymers accelerate reannealing of DNA in solution. Although such compounds should also accelerate hybridization of probes to immobilized DNA, the net effect of using such polymers in two-phase hybridizations was not easy to predict because self-annealing of the probe and hybridization to the DNA-paper could have been accelerated to different extents and because the backgrounds might have increased. To test the use of dextran sulfate, DNA from a hamster cell mutant with about 7 times the number of wild-type genes for the multifunctional protein was digested with *Eco*RI, fractionated on an agarose gel, and transferred to DBM-paper. Identical strips were hybridized with the same amount of nick-translated probe by using different concentrations of dextran sulfate (Fig. 4). The signal obtained after a fixed time of hybridization increased dramatically with increasing concentration of the polymer. After 2 hr of hybridization in the presence of 10% dextran sulfate the signal obtained was 3–4 times greater than that after 72 hr in its absence (compare tracks b and c of Fig. 5). The signal-to-background ratio was higher for short hybridizations with dextran sulfate than for long hybridizations without the polymer. Long hybridizations with dextran sulfate under the conditions used for Fig. 5 resulted in unacceptably high backgrounds (Fig. 5, track e). However, the background was decreased substantially when the DNA-paper was treated with hybridization buffer containing 5 times the usual concentration of Denhardt's reagent before hybridization (see *Procedure*).

How Dextran Sulfate Accelerates Two-Phase Hybridizations. The rate of reassociation of DNA in solution is increased

FIG. 4. Effect of dextran sulfate concentration on hybridization of a nick-translated probe to DNA-paper. DNA (1 μg/track) obtained from a mutant with a 7-fold increase in the concentrations of both multifunctional protein and mRNA and approximately the same increase in the number of genes was cleaved with *Eco*RI, fractionated on a 0.7% agarose gel, and transferred to DBM-paper. Identical strips of DNA-paper were hybridized for 16 hr in the presence of the indicated concentrations of dextran sulfate (0, 5, and 10%) and 5×10^6 cpm of nick-translated probe (1×10^6 cpm/ml, 5×10^7 cpm/μg). The washed filters were autoradiographed for 10 hr.

approximately 10-fold by 10% sodium dextran sulfate 500 (7). However, hybridization of nick-translated probe to DNA-paper is about 100 times faster with the polymer (compare tracks b and c of Fig. 4). The effects of dextran sulfate on rates of hybridization of single-stranded and double-stranded probes to DNA-paper reveal much about the mechanism of the rate enhancement. Labeled single-stranded φX174 viral DNA, average length approximately 250 nucleotides, and nick-translated double-stranded φX174 replicative form DNA were hybridized to φX174 DNA-paper in the presence or absence of 10% dextran sulfate (Table 1). Three to four times more single-stranded

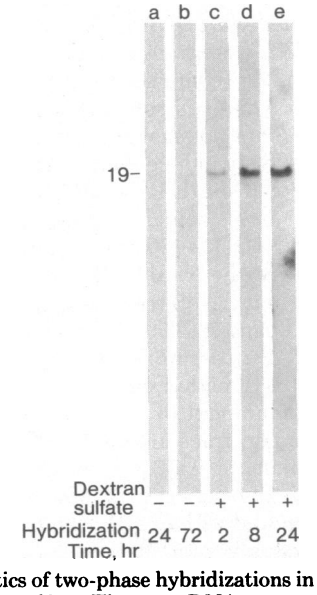

FIG. 5. Kinetics of two-phase hybridizations in the presence and absence of dextran sulfate. The same DNA-paper strips described in Fig. 4 were hybridized without or with 10% dextran sulfate using 3×10^6 cpm of nick-translated probe (6×10^5 cpm/ml, 5×10^7 cpm/μg). The washed strips were autoradiographed for 16 hr. In this experiment the buffer used in the prehybridization step did not contain 5 times concentrated Denhardt's reagent (see *Procedure*); consequently, the strips hybridized for long periods with the polymer have a high background.

Table 1. Effect of dextran sulfate on the hybridization of single-stranded or double-stranded probes to DNA-paper

	Single-stranded probe		Nick-translated probe	
10% dextran sulfate	−	+	−	+
cpm hybridized	110	330	70	860
Fold enhancement by dextran sulfate	—	3	—	12

The DNA of bacteriophage ϕX174 replicative form was cleaved with *Pst* I at its unique site, fractionated on a 0.7% agarose gel, and transferred to DBM-paper. Identical strips, each with approximately 1 ng of the *Pst* I fragment, were hybridized for 12 hr with 0.5–1 × 10^6 cpm (1–2 × 10^7 cpm/μg, 2–4 × 10^5 cpm/ml) of 5′-labeled single-stranded fragments of ϕX174 viral DNA or with fragments of nick-translated double-stranded replicative form DNA having approximately the same concentration and specific activity. The washed papers were autoradiographed for 4 hr, the bands were excised, the hybridized probes were eluted with 0.4 M NaOH, and the amount of Cerenkov radiation was determined in a liquid scintillation counter. A background of 40 cpm was subtracted from each value.

probe binds to the DNA-paper in 12 hr in the presence of dextran sulfate than binds in its absence. Wetmur (7) has attributed the 10-fold increase in reassociation rate caused by 10% dextran sulfate in homogeneous solution to exclusion of DNA from the volume occupied by the polymer—i.e., to concentration of the DNA. For a bimolecular reaction, an increase in rate of 10-fold corresponds to an increase in the concentration of each component of about 3-fold. In a two-phase hybridization, only the soluble component can be concentrated, so the observed 3- to 4-fold increase with single-stranded probe is just what would be anticipated. Double-stranded probe gave a dramatically different result (Table 1). In this case the rate in the presence of dextran sulfate was more than 12 times the rate in its absence. In the experiment shown in Fig. 5, a different probe was used and the increase was about 100-fold. If the major effect of dextran sulfate is to accelerate the formation of probe networks, as discussed next, the length of the probe will probably affect the degree of enhancement.

Nick-translation generates randomly cleaved molecules so that, after denaturation, partially complementary fragments can reanneal to form a partial duplex that retains single-stranded regions. Continued reannealing should lead to extensive networks that always retain single-stranded regions. Dextran sulfate should accelerate both the formation of such networks and their eventual hybridization to DNA-paper. Because the immobilized DNA has also been cleaved randomly, hybridization to a single-stranded probe will leave single-stranded regions of probe or paper-bound DNA available for further hybridization. Large networks of probe can become attached to DNA-paper all at once or can be built up more gradually. In confirmation of this model, adding unlabeled, denatured fragments of ϕX174 replicative form DNA to labeled ϕX174 single-stranded probe increases the rate of hybridization of the labeled DNA to DNA-paper in the presence of dextran sulfate (data not shown). Networks comprised of labeled and unlabeled molecules must have hybridized with the immobilized DNA in this case. Further confirmation of the model might be obtained by showing that the amount of probe hybridized can exceed the amount of immobilized DNA. However, this experiment may be difficult because only a minor fraction of the immobilized DNA seems to be available for hybridization (see ref. 19).

Other Uses of Dextran Sulfate. Hybridization of nick-translated probe to RNA-paper (5) is also accelerated greatly by 10% dextran sulfate (unpublished results). Dextran sulfate also increases the rates of *in situ* hybridizations (20) used to locate specific sequences in polytene chromosomes of *Drosophila* (M. Wolfner, personal communication) and detection of recombinant mammalian viruses in plaques (21) (R. Mulligan, personal communication). Detection of recombinant molecules in the plaque-filter (22) and colony-filter (23) methods should also be facilitated. Dextran sulfate should also accelerate hybridization of RNA to DNA-cellulose (4) or DNA-paper (19), decreasing the time required for RNA purification about 1:3.

Conclusions. The techniques described in this paper should improve both the speed and sensitivity with which genes can be located in digests of DNA. Nearly quantitative transfer of DNA to DBM-paper makes it possible to analyze quantitatively the abundance of specific restriction fragments. Potential clinical applications derive from the fact that DNA-paper is very stable and can be reprobed many times. For example, one could digest with various restriction endonucleases very small samples of DNA from a patient and make an essentially permanent transfer record of the separated fragments. Such a transfer could be tested repeatedly with different probes and could be stored for long periods.

We thank Dr. Ronald W. Davis and Thomas St. John for helpful suggestions, and Thomas St. John, Mariana Wolfner, and Richard Mulligan for allowing us to quote unpublished results. This investigation was supported by Grant CA 17287 awarded to G.R.S. by the National Cancer Institute and by California Division-American Cancer Society Senior Fellowship D-384 awarded to G.M.W.

1. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
2. Mears, J. G., Ramirez, J., Leibowitz, D., Nakamura, F., Bloom, A., Konotey-Ahula, F. & Bank, A. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 1222–1226.
3. Kan, Y. W. & Dozy, A. M. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5631–5635.
4. Noyes, B. E. & Stark, G. R. (1975) *Cell* **5,** 301–310.
5. Alwine, J. C., Kemp, D. J. & Stark, G. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5350–5354.
6. Reiser, J., Renart, J. & Stark, G. R. (1978) *Biochem. Biophys. Res. Commun.* **85,** 1104–1112.
7. Wetmur, J. G. (1975) *Biopolymers* **14,** 2517–2524.
8. McDonell, M. W., Simon, M. N. & Studier, F. W. (1977) *J. Mol. Biol.* **110,** 119–146.
9. Rigby, P. W. S., Dieckman, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
10. Wahl, G. M., Padgett, R. A. & Stark, G. R. (1979) *J. Biol. Chem.* **254,** in press.
11. Donoghue, D. J. & Sharp, P. A. (1977) *Gene* **1,** 209–227.
12. Maxam, A. & Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 560–564.
13. Denhardt, D. (1966) *Biochem. Biophys. Res. Commun.* **23,** 641–652.
14. Shank, P. R., Hughes, S. H., Kung, H.-J., Majors, J. E., Quintrell, N., Guntaka, R. V., Bishop, J. M. & Varmus, H. E. (1978) *Cell* **15,** 1383–1395.
15. Lis, J. T., Prestidge, L. & Hogness, D. S. (1978) *Cell* **14,** 901–919.
16. Kochetkov, N. K. & Budovskii, E. I. (1972) *Organic Chemistry of Nucleic Acids* (Plenum, London), Part B, pp. 477–532.
17. Gillespie, D. & Spiegelman, S. (1965) *J. Mol. Biol.* **12,** 829–842.
18. Wetmur, J. G. & Davidson, N. (1968) *J. Mol. Biol.* **31,** 349–370.
19. Stark, G. R. & Williams, J. G. (1979) *Nucleic Acids Res.* **6,** 195–203.
20. Pardue, M. L. & Gall, J. G. (1969) *Proc. Natl. Acad. Sci. USA* **64,** 600–604.
21. Villareal, L. P. & Berg, P. (1977) *Science* **196,** 183–185.
22. Benton, W. D. & Davis, R. W. (1977) *Science* **196,** 180–182.
23. Grunstein, M. & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3961–3965.