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High Molecular Weight DNA from Fixed Cytogenetic Preparations

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SUMMARY

Cell pellets that have been stored in routine clinical cytogenetic fixative were studied for the presence of intact DNA. A method for the isolation of high molecular weight DNA from fixed cytogenetic preparations of human leukocytes, bone marrow, and cell hybrid cultures is presented. DNA preparations from fixed pellets were cleaved with restriction enzymes, transferred to nitrocellulose filters after agarose gel electrophoresis, and hybridized to radiolabeled probes to demonstrate that fixed cell pellets could yield DNA of sufficient quality for Southern blot hybridization analysis. This protocol may be useful for molecular analysis of DNA from fixed cell pellets of patients who are unavailable for additional sampling.

INTRODUCTION

Isolation of cloned DNA probes for human genetic studies has opened up a range of new clinical diagnostic capabilities based upon direct analysis of genomic DNA samples, usually from peripheral blood leukocytes or chorionic villus biopsies [1–4]. In addition to analysis of single-gene Mendelian disorders, cloned oncogene probes have been useful in unraveling the molecular basis of chromosome translocations observed in some human [5] and murine [6] malignancies.

These techniques require fresh tissues, cultured cells, or fresh frozen tissues

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from affected individuals. Cytogenetic diagnostic laboratories often save fixed cell pellets for future studies [7]. These are usually treated with a 3:1 (v/v) mixture of methanol and glacial acetic acid to fix the mitotic cells and stored in fixative in a refrigerator or freezer. In some instances, especially in cytogenetic facilities associated with hematology/oncology divisions, the fixed samples represent rare specimens from patients with unusual chromosomal abnormalities. Often, the original patient is not available for further studies.

During follow-up studies of the pre-B cell acute lymphocytic leukemia translocation between human chromosome 1 and 19 [8], it was noted that high molecular weight DNA could be obtained from fixed cytogenetic preparations [9]. Since this finding was unanticipated and may be useful to others who wish to study DNA from fixed cell pellets, we report our results here.

MATERIALS AND METHODS

Isolation protocols followed published methods [10, 11]. For unfixed or frozen viable samples, cells were rinsed once in Hank's balanced salt solution, then resuspended in 50 mM Tris-Cl, 10 mM NaCl, 10 mM EDTA, pH 8 (TNE), made to 0.7% w/v sodium dodecyl sulfate after suspension of cells, and then made to a final concentration of 100 μg/ml predigested proteinase K. The cell lysates were digested at 37°C for 4–12 hrs and extracted twice with TNE-saturated phenol, pH 8. This was followed by two extractions with a 24:1 (v/v) mixture of chloroform/isoamyl alcohol, and the aqueous DNA was loaded into dialysis bags for dialysis at 4°C against 10 mM Tris-Cl, 1 mM EDTA, pH 8 (TE). The DNA samples were concentrated by reverse dialysis against polyethylene

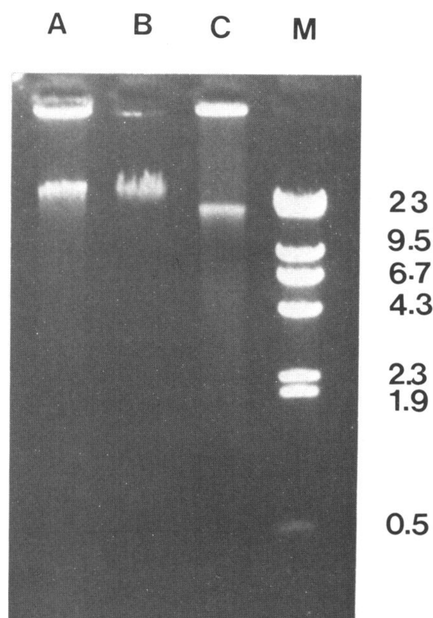

FIG. 1.—Agarose gel of uncleaved DNA from (*A*) fixed bone marrow mitotic cell pellet stored 1.5 yrs, (*B*) fresh bone marrow, (*C*) fixed bone marrow mitotic cell pellet stored 3 wks, and (*M*) marker tract containing *Hin*dIII-cleaved λDNA. Marker size fragments are given *to the right* in kilobases.

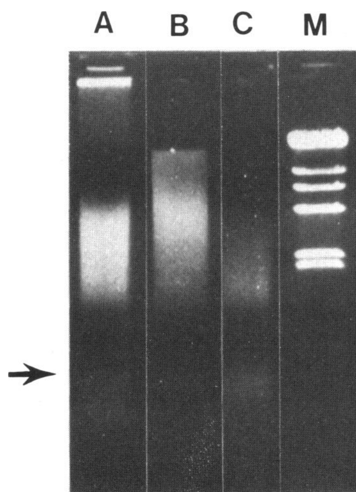

FIG. 2.—Lanes containing DNA samples cleaved with *Eco*RI and separated on a 0.6% agarose gel. (*A*) Fixed bone marrow stored 1.5 yrs, (*B*) fresh bone marrow, (*C*) fixed bone marrow mitotic pellet stored 3 wks, and (*M*) marker track as in figure 1. *Arrows* show *Eco*RI repeat DNA bands in genomic DNA.

glycol 8000 (Sigma) to greater than 200 μg/ml, and then dialyzed again against TE overnight at 4°C. DNA was similarly extracted from fixed cell pellets except that the cell pellets were rinsed once at 4°C in TNE prior to resuspension in the same buffer and detergent lysis.

DNA samples were analyzed by gel electrophoresis on 0.6% (w/v) agarose gels in Tris-borate buffer as reported [11]. DNA samples were digested to completion with restriction endonucleases according to supplier instructions except that for *Eco*RI digestion spermidine and RNAse A were added to 10 mM and 50 μg/ml, respectively.

Southern blot analysis was carried out as reported [12] by transfer of cleaved, electrophoretically separated DNA fragments to BA85 nitrocellulose filters (Schleicher & Schuell). Prehybridization and aqueous hybridization with probes labeled by nick-translation [13] at 10 ng/ml (specific radioactivity $2–4 \times 10^8$ dpm/μg) was performed with dextran sulfate to 10% (w/v) at 65°C for 14 hrs. Wash conditions for blot analyses with the human c-*myb* probe [14] were at high stringency as defined [15]. Autoradiography of washed filters was accomplished with intensifier screens at −20°C on Kodak XAR5 film.

RESULTS

Figure 1 shows an ethidium bromide-stained agarose gel containing DNA from a series of bone marrow samples. Although very slight degradation can be seen in the fixed pellets (lanes A and C), most DNA appears below the wells and in a band greater than 23 kilobases (kb) in molecular weight. Aliquots of the same DNA were analyzed by gel electrophoresis after cleavage with *Eco*RI digestion (fig. 2).

Figure 3 shows a similar experiment in which peripheral leukocyte DNA was cleaved with *Eco*RI directly (lane B) and after treatment of the cells with the standard cytogenetic fixation with anhydrous reagents (lane C). These DNAs

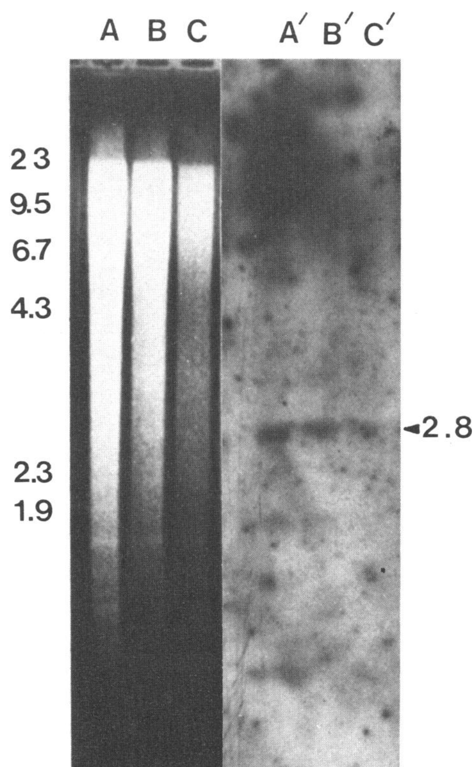

FIG. 3.—Agarose gel (*left*) and Southern blot filter probed with human c-*myb* probe (*right*). The genomic DNA samples were cleaved with *Eco*RI. (*A*) Normal placental DNA control, (*B*) direct peripheral blood leukocyte DNA, (*C*) DNA from leukocytes fixed with methanol/acetic acid. The molecular weight markers as in figure 1 are shown *to the left*. *The arrow* shows a 2.8-kb human c-*myb* band [15].

were transferred to nitrocellulose filter paper and probed with radiolabeled human c-*myb* probe (lanes A′ through C′). As shown at the arrow, the typical c-*myb* *Eco*RI band of about 2.8 kb appears in both the unfixed samples (placental control in lane A and A′, direct unfixed leukocytes in lanes B and B′) and in the fixed sample of lanes C and C′. Thus, it is possible to detect an intact 2.8-kb human c-*myb* band even in cell pellets that have been treated with methanol/acetic acid fixative.

The same protocol was used to isolate DNA from four somatic cell hybrid cell pellets. After slides were prepared for G-banding analysis, the remaining fixed cell pellets from one T75 culture flask each were rinsed in TNE and lysed for DNA isolation. An agarose gel electrophoresis experiment (fig. 4) indicated that these fixed cell pellets contained high molecular weight DNA. However, a low molecular weight band was also present. To determine whether this was degraded DNA, the gel was incubated in Tris-borate electrophoresis buffer containing 100 μg/ml preboiled RNAse A for 2 hrs at 37°C (fig. 4, lanes A′–D′). Since the low molecular weight band was sensitive to RNAse A, it contained

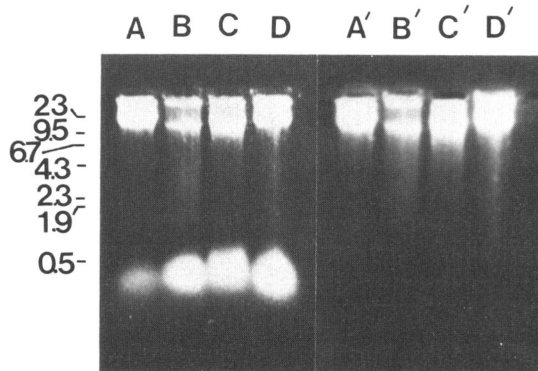

FIG. 4.—Uncleaved genomic DNA isolated from fixed mitotic cell pellets from somatic cell hybrids (*A*) A9 × CH 13-4, (*B*) A9 × CH 26, (*C*) A9 × CH × 1-14 cl.2, and (*D*) A9 × CH 13-4. Agarose gel *to the left;* gel after RNAse treatment *to the right*. Molecular weights determined as in figure 1 are shown *to the left* in kilobases.

RNA, not degraded DNA. These results show that, in fixed cell pellets from cultures of four somatic cell hybrids, both high molecular weight DNA and RNA were present.

To determine whether fixed cell pellets stored over several months' duration would yield DNA sufficiently intact for Southern blot analysis, two fixed bone marrow samples of 0.2 ml packed cell volume were prepared as above. The DNA from pellets stored 125 and 441 days after fixation was cleaved with *Eco*RI, transferred to nitrocellulose, and probed with a cDNA probe for the insulin receptor (INSR) [16]. The results appear in figure 5. Lane m refers to end-labeled λDNA cleaved with *Hin*dIII as a molecular weight standard with sizes in kilobases to the left. Lane 125 contained *Eco*RI-cleaved DNA and shows fragments typical of the INSR locus. The DNA from lane 441 was isolated as above from a fixed bone marrow cell pellet that had been stored at −20°C in fixative for 441 days. The constant 11 and 3.1 kb INSR bands are clearly visible in both lanes with approximate molecular weights to the right. In addition, a variable band is noted at either 5.8 kb (lane 441) or at 6.5 kb (lane 125). This may represent a restriction fragment length polymorphism at the INSR locus [3]. This experiment shows directly that fixed cell pellets stored up to 441 days in methanol-acetic acid fixative at −20°C can yield high molecular weight DNA that can be analyzed in standard Southern blot experiments.

DISCUSSION

We have shown that fixed cell pellets can yield high molecular weight DNA that can be cleaved with restriction enzymes and analyzed by Southern blot hybridization experiments. These experiments demonstrate that fixed cell pellets stored up to 441 days in fixative at −20°C can still yield clear Southern blot results. Although fixed cell pellets may not be optimal starting material for DNA extractions, in some instances, the pellets represent the only source of

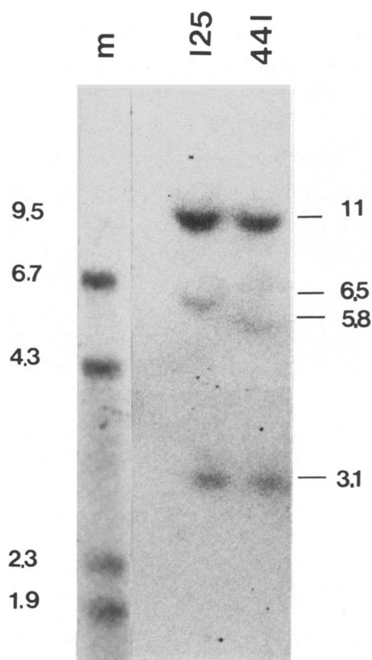

FIG. 5.—Autoradiograph of hybridization experiment in which DNA from fixed bone marrow samples was isolated after storage of pellets in fixative for 125 days (*lane 125*) and 441 days (*lane 441*). This filter was hybridized with a cDNA probe from the insulin receptor locus [16]. Molecular weight standards are end-labeled λDNA cleaved with *Hin*dIII shown in kilobases *to the left* (*lane m*).

DNA from patients or cell culture material previously studied by classical cytogenetic methods.

In the course of this work, we had success with some but not all fixed cell pellets. The critical parameters that distinguish those pellets containing intact DNA from those that contain degraded DNA are still under investigation. Among those variables that may prove important are: (1) the exclusion of water from fixation reagents, (2) minimal exposure of fixed pellets to temperatures above 0°C, (3) the use of large fixed pellets (greater than 0.2 ml packed volume) for DNA isolation, and (4) the mitotic index of the fixed cell pellet. The first may be important because depurination of DNA by acid is known to be a hydrolysis reaction [17, 18]. The second consideration applies to keeping pellets on ice during fixation and immediately storing pellets at −20°C or below. In our limited experience, the results are better with large than with small pellets. It is not known whether the high molecular weight DNA from chromosomes at different stages of the cell cycle is differentially sensitive to degradation as a result of cytogenetic fixation procedures.

Workers in several areas of genetic analysis may find these results useful. Many laboratories routinely store fixed cell pellets in the event that this mate-

rial may be needed for future cytogenetic and in situ hybridization studies. These results will allow reevaluation of previously characterized karyotype pellets with DNA probes. This may apply for family studies using DNA markers or in molecular analyses of cytogenetically characterized leukemic bone marrow fixed cell pellets. In the latter instance, DNA from fixed cell pellets would be available for analysis with cloned oncogene probes. In many patients with hematological malignancies, the death of the patient prevents access to fresh tissue samples for DNA analysis. Our method may provide an alternative source of DNA.

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