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# Isolation and Characterization of a Near-Haploid Human Cell Line

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**Mammalian somatic cells are usually diploid. Occasional rare human tumors have been shown to have a hypodiploid karyotype. We have isolated a near-haploid subclone (P1-55) from a heterogeneous human leukemia cell line, KBM-7. These near-haploid cells have approximately half the human diploid DNA content and have a haploid karyotype except for a disomy of chromosome 8 (25, XY, +8, Ph$^+$). This cell line maintains a majority of cells with a near-haploid karyotype for at least 12 weeks in culture. By serial subcloning, we have isolated near-haploid subclones that maintain ploidy for at least 8 months in culture. Near-haploid cells can also be efficiently isolated from mixed ploidy cultures by size selection. The availability of this human near-haploid cell line should facilitate the genetic analysis of cultured human cells.** © 1999 Academic Press

***Key Words:*** **haploidy; KBM-7; somatic cell genetics.**

## INTRODUCTION

One factor limiting the genetic analysis of mammalian cells in culture is that these cells have a diploid or higher number of chromosomes. As a result, the phenotype of a loss of function mutation on one chromosome is not expressed unless the corresponding allele on the other chromosome is also mutated. In contrast, bacteria and yeast are readily amenable to genetic analysis in part because they are haploid [1]. Previously, karyotypically stable cell lines which maintain a near-haploid karyotype have been isolated from amphibians and insects [2, 3]. There are currently no similar cell lines available for mammals. However, near-haploid karyotypes have been documented in rare human tumors and leukemias [4]. Thus, it seems that near-haploid mammalian cells can be viable.

Previously, Andersson *et al.* [5] established a heterogeneous (mixed ploidy) cell line (KBM-7) from the bone marrow of a patient with a near-haploid chronic myeloid leukemia. Though these cultures were initially slightly greater than 50% near-haploid, cells with a diploid or greater DNA content rapidly overgrow the KBM-7 cultures, rendering this cell line unsuitable for somatic cell genetics.

The karyotype of KBM-7 indicated that the near-haploid cells in the culture had disomies for both chromosomes 8 and 15 [5, 6]. However, leukemic cells isolated directly from this patient had two distinct near-haploid karyotypes. Some cells had disomies of both chromosomes 8 and 15, others had only a disomy of 8. Analysis of the leukemic cells from the patient also indicated the presence of a large percentage of near diploid cells with a karyotypic duplication of the 26, XY, +8, +15 near-haploid clone [5]. However, there were no near-diploid cells karyotyped that represented a duplication of the haploid clone with only a disomic 8. This observation suggested that the +8 near-haploid clone might be more karyotypically stable than the +8, +15 clone found in KBM-7.

Here we report the isolation and characterization of a near-haploid cell line which has only a disomy of chromosome 8 from the heterogeneous human leukemia cell line KBM-7. This cell line remains karyotypically stable for many weeks in culture and near-haploid suclones can be repeatedly isolated from this population of cells, allowing for the continuous maintenance of near-haploid cells in culture. These properties should make this cell line useful for somatic cell genetics.

## MATERIALS AND METHODS

*Cell culture and DNA content analysis.* KBM-7 and its derivatives were routinely cultured in Iscove's medium + 15% fetal calf serum at 37°C in an atmosphere of 5–10% $CO_2$. Cultures were passaged every 3–5 days at a density not less than $5 \times 10^5$ cells/ml. For DNA content analysis, cells were stained with 9 $\mu$M Hoechst 33342 (Molecular Probes, Eugene, OR) and 0.3 $\mu$g/ml Dio-C5-3(3,3-dipentyloxa-carbocyamine) in culture medium at 37°C for 60 min and the DNA content per cell was estimated by measuring the fluorescence using a UV laser at 70–200 mW power in a Becton Dickinson FACStar Plus cell sorter [7].

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*Cell cloning.* Haploid cell clones were isolated by cloning in soft agar. First, a 5-ml bottom layer of 0.8% agarose in Iscove's medium containing 15% fetal calf serum was poured into 60-mm dishes. Then the KBM-7 cells (100–10,000 cells) were mixed with 5 ml of medium containing 0.37% low-melting-point (LMP) agarose at 37°C and overlaid on top of the bottom agarose layer. The LMP agarose layer was rapidly solidified by placing ethanol-soaked paper towels on the plates for 15 min. The plates and the paper towels were separated by aluminum foil. Then the plates were incubated at 37°C and 5–10% $CO_2$ for 2–3 weeks. Cells were fed by overlaying 4 ml of medium in 0.37% LMP agarose every 7 days. When the colony size reached approximately 1 mm in diameter, colonies were picked and expanded in 1 ml medium in a 24-well plate and the haploid clones were identified by FACS analysis after 2–4 weeks of culture.

*Karyotyping.* For karyotyping, P1-55 cells 12–14 weeks after subcloning were harvested following incubation with 60 $\mu$g/ml bisbenzimid (Sigma Chemical Co., St. Louis, MO) and $2.5 \times 10^{-5}$ M ethidium bromide (Sigma Chemical Co.) for 2.5 h and 0.07 $\mu$g/ml colcemid (Gibco) for 1 h. Cells were then swelled in 0.56% KCl at room temperature for 8 min. Cells were pelleted and a −20°C mixture of glacial acetic acid and methanol 1:3 (v/v) was added dropwise without agitation in order to prevent chromosomal losses resulting from metaphase plate rupture and overspreading. Agitation was found to preferentially rupture the near-haploid metaphases. Chromosomes were GTG-banded as described [8].

## RESULTS

*Isolation of P1-55.* Consistent with the work of Andersson *et al.* [5], we found the partially near-haploid heterogeneous KBM-7 cell line to be highly karyotypically unstable. First-passage cultures from this cell line show that approximately 55% of the cells have a near-haploid DNA content by FACS analysis (see Fig. 1A). However, when these cultures are further passaged, cells with a diploid or greater DNA content quickly overgrow the culture (see Fig. 1B). This karyotypic instability renders the KBM-7 cell line unsuitable for somatic cell genetics.

To determine whether more stable near-haploid cells could be isolated from the KBM-7 cultures, first-passage KBM-7 cells were subcloned in soft agar. Sixty-two subclones were isolated and characterized by FACS analysis for DNA content. Of these 62 cell lines, five showed a near-haploid DNA content. The percentage of clones that were near-haploid was less than their representation in the starting population because near-haploid cells were found to have a lower cloning efficiency in soft agar than near-diploid cells (data not shown). Three of these haploid subclones quickly evolved into higher ploidies upon further cultivation. The most stable of the near-haploid subclones, which we call P1-55, maintains a near-haploid DNA content in the majority of cells for up to 12 weeks in culture. The G1 DNA content of this cell line was 51 ± 2% of second-passage G1 human primary fibroblasts as measured by Hoechst 33342 dye staining (Fig. 1C). By the same method, the G1 DNA content of the haploid cells in the KBM-7 line was within 2% of the DNA content of G1 phase P1-55 cells. P1-55 DNA content determined by comparison to primary human lymphocytes was slightly higher–approximately 60%. The difference is likely due to differential dye uptake and stainability of growing fibroblasts and quiescent lymphocytes. The expected value based on the karyotype (see below) would be 53% [9].

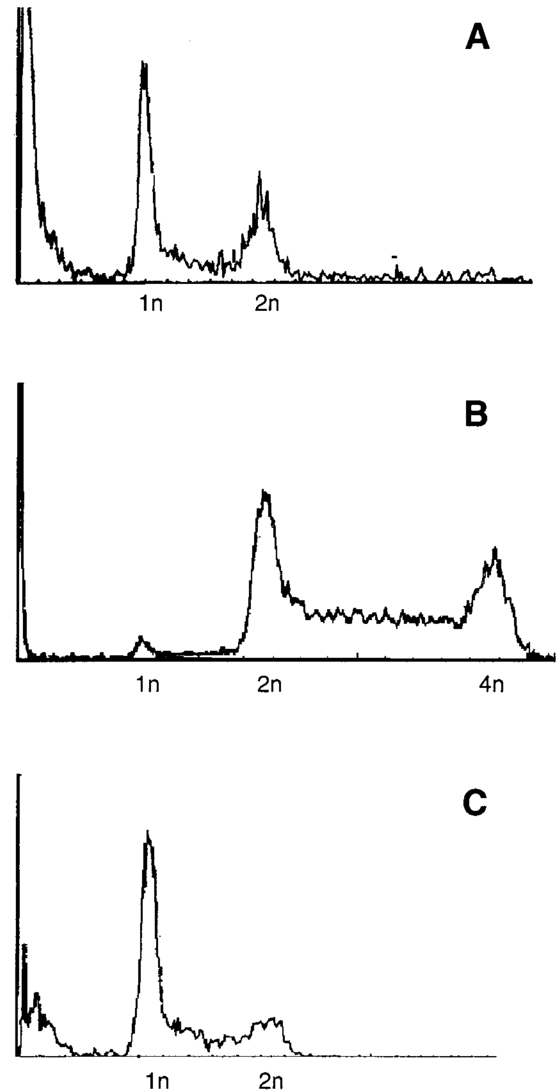



**FIG. 1.** Estimation of ploidy and DNA content of KBM-7 and the near-haploid clone P1-55 by flow cytometric analysis. Cells were analyzed for DNA content as described under Materials and Methods. Fluorescence intensity scales varied slightly between experiments. Human primary fibroblasts were used as standard to determine the relative fluorescence intensities of $1n$, $2n$, and $4n$ quantities of the human haploid DNA content. The positions of the $1n$, $2n$, and $4n$ peaks are indicated. (A) KBM-7 at first passage. (B) KBM-7 after 20 days in culture. (C) The near-haploid subclone P1-55 at 4 weeks after subcloning from soft agar.

*Karyotype.* At 12 to 14 weeks in culture after subcloning, cells from the P1-55 cell line were analyzed for karyotypes. A typical near-haploid karyotype is shown

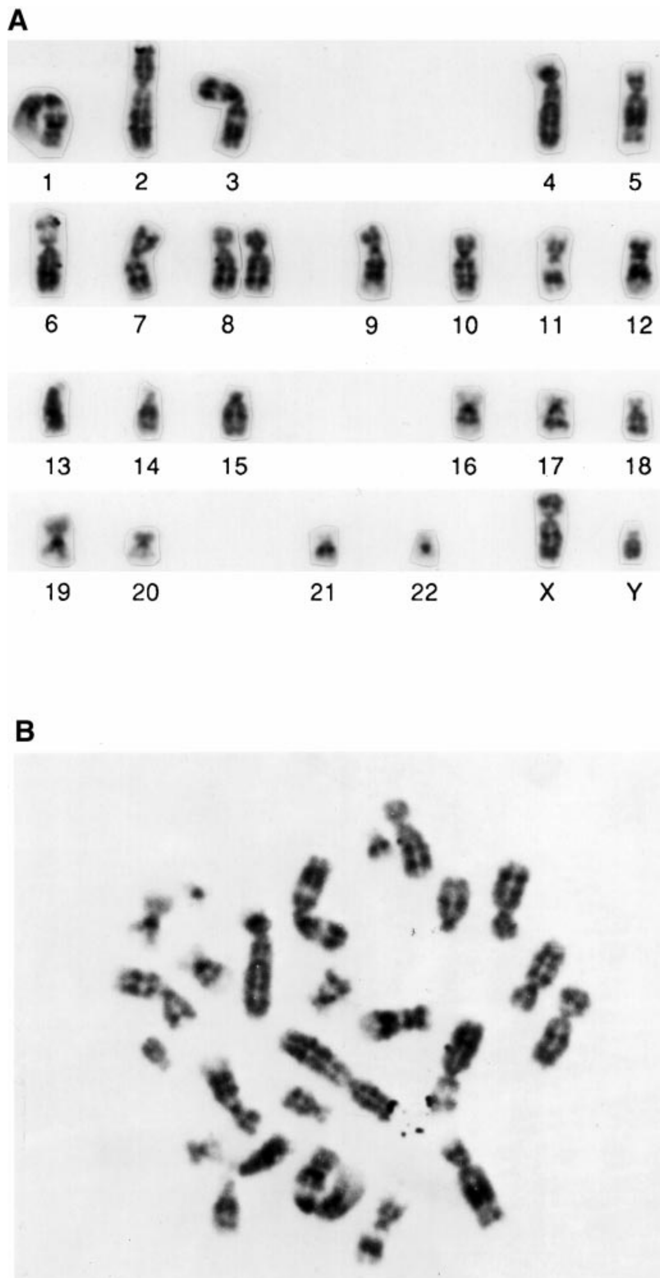



**FIG. 2.** Karyotype of the near-haploid clone P1-55. The PI-55 cell line was karyotyped as described under Materials and Methods. (A) A representative karyotype showing disomy of chromosome 8 and the t(9q;22q) Philadelphia chromosome in a cell with 25 chromosomes. (B) A near-haploid cell in metaphase.

in Fig. 2. A summary of these karyotypes is given in Table 1. The most frequent karyotype observed was that of cells with 25 chromosomes with monosomies at every chromosome except chromosome 8, which was disomic (25, XY, +8, $Ph^+$). A variation of this karyotype was also found that was identical except that it lacked the Y chromosome, which is frequently lost in leukemic cells [10]. Except for the Ph translocation t(9q;22q) which was present in all cells, no structural rearrangements involving two chromosome breakpoints in the same metaphase were found in the P1-55 cells. The P1-55 karyotype is concordant with the presumably more stable near-haploid cells found in bone marrow cells of the patient from which KBM-7 was derived, but differs from the one reported for KBM-7 itself by the monosomy for chromosome 15 and the presence of the Y chromosome [5]. Thus, it is likely that the cells with only a disomy for chromosome 8 were a small fraction of the first-passage KBM-7 population and were rapidly overgrown by the karyotypically less stable 26, XY, +8, +15 clone (see discussion below and Fig. 3).

The karyotype analysis also reveals that the P1-55 cell line is not homogeneous with respect to ploidy. Several near-diploid cells were found which were apparent duplications of the near-haploid clones with disomic Philadelphia chromosomes and tetrasomies of chromosome 8 (Table 1). In addition, some near-tetraploid metaphases were observed. Two of these cells were karyotyped and were found to be almost exact karyotypic duplications of the diploid cells. Consistent with the FACS analysis, no clonal karyotypes were found to have intermediate numbers of chromosomes between 1, 2, and $4n$. Because these near-diploid and near-tetraploid cells have karyotypes that are even multiples of the predominate haploid cells, it is likely that they were formed in a single event by either endoreduplication or cell fusion rather than gradually. Since no triploid cells were observed, it is unlikely that either the diploid or tetraploid cells arose by cell fusion.

*SSR polymorphism analysis.* The near-haploid karyotype of P1-55 does not imply that every genetic locus not on chromosome 8 is haploid, since small duplications and translocations cannot be identified at this level of resolution. Nevertheless, since the overall DNA content of these cells is ~52% of human diploid fibroblasts, the great majority of loci are likely to be haploid. Simple sequence (CA) repeat polymorphisms were examined for several arbitrarily selected chromosomes and chromosome 8, to determine whether a molecular analysis of zygosity would be consistent with the near-haploid karyotype and whether the disomic chromosome 8 was homo- or heterozygous. (See Table 2.) None of the loci tested on chromosomes 3, 10, and 15 from this cell line were found to be heterozygous. This result is consistent with the karyotype showing that these chromosomes were monosomic. In contrast, all (4/4) of the chromosome 8 loci tested were heterozygous. This result also indicates that the disomy of chromosome 8 did not arise by chromosome duplication. Loci on other chromosomes were not examined.

*Stability.* Although the P1-55 cell line was clonally derived, with increasing time in continuous culture, the percentage of the cells that had a duplicated set of

**TABLE 1**

Karyotype Analysis of the P1-55 Cell Line

| No. of cells | No. of chromosomes | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | X | Y |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | 24 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 50 | 25 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 6 | 46 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 4 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 0 |
| 5 | 49 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 4 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 |
| 7 | 50 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 4 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| 1 | 98 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 7 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 1 | 98 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 7 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 |

*Note.* Karyotypes were determined by GTG banding of P1-55 as described under Materials and Methods. One hundred thirty-one metaphases with good spreading and banding patterns were photographed and karyotyped. A karyotype was considered to be clonal within the population if at least five cells were observed with the same karyotype. Unique karyotypes were found for 43 metaphases with chromosome numbers ranging from 22 to 49. The random distribution of chromosome losses (except for preferential loss of X and Y) in these spreads likely indicates that these karyotypes were not clonal populations, but were more likely generated by rupture of near-diploid metaphase plates during preparation. Since only high-quality chromosome spreads were selected for complete analysis, table does not quantitatively reflect the relative distribution of near-haploid and near-diploid cells in the culture. In addition, the quantitative distribution of cell karyotypes is further skewed by the fact that optimal fixation conditions differed for near-haploid and near-diploid cells.

chromosomes increased. (See Fig. 3.) However, even after 12 weeks in culture, more than 50% of the cells had a near-haploid DNA content. This number is actually an underestimate of the percentage of the haploid cells in the population since haploid cells in G2/M cannot be distinguished from diploid cells in G1 by DNA content measurement in the FACS. Nevertheless, the near-diploid cells must have a slight growth advantage over the near-haploid ones. However, since the majority of cells remain haploid for long periods of time in culture, the incidence of spontaneous increase in ploidy in these cultures must be relatively low. In addition, we have found that culture conditions and subcultivation protocols can affect the rate of diploid outgrowth, but even under identical cultivation protocols other subclones of KBM-7 that were examined evolved into higher ploidies much more quickly than P1-55. The least stable of these (P1-32) was as unstable as KBM-7 and was found to be disomic for both chromosomes 8 and 15 and lacked Y as was reported for the original KBM-7 line [5] and the KBM-7/B5 subclone [6]. (See Fig. 3.) This cell line also had a faster doubling time than P1-55 (data not shown). These properties of P1-32 could account for the rapid diploidization of KBM-7 and the predominance of cells with the +8, +15 karyotype in the KBM-7 line.

**TABLE 2**

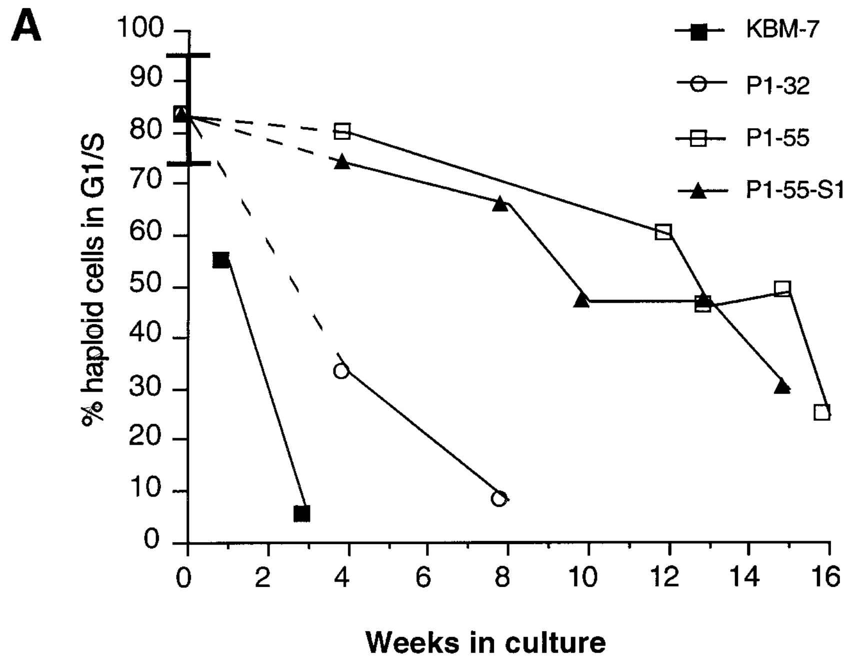
Heterozygosity Analysis of P1-55 Using Simple Sequence Repeat Polymorphisms

| Chromosome No. | Zygosity (heterozygous/number loci tested) | | |
|---|---|---|---|
| | KBM-7 | P1-55 | control |
| 3 | 0/4 | 0/4 | 2/4 |
| 8 | 4/4 | 4/4 | 3/4 |
| 10 | 0/4 | 0/4 | 4/4 |
| 15 | 2/4 | 0/4 | 4/4 |

*Note.* Markers of CA repeat polymorphisms from the indicated chromosomes were analyzed as described by Hudson *et al.* [31]. HeLa cell DNA was used as a control for chromosome 3 markers and CEPH cell DNA was used as a control for all the chromosome polymorphisms examined. Loci examined for polymorphisms were as follows: chromosome 3—D3S 1209, D3S 1212, D3S 1215, D3S 1216; chromosome 8—D8S205, D8S206, D8S207, D8S208; chromosome 10—D10S172, D10S173, D10S174, D10S175; chromosome 15—D15S98, D15S100, D15S101, D15S102. The probability of detecting polymorphisms on any one of the analyzed chromosomes for a given human diploid cell with these markers is >99%.

Despite the tendency to drift toward higher ploidies, haploid subclones (P1-55-S1) could be reisolated from the mixed-ploidy cultures of P1-55 and maintained in culture for long periods with a high percentage of near-haploid cells (Fig. 3). Further subcloning of P1-55-S1 has yielded haploid cells with even better stability in culture as shown in Fig. 3B. These cells can be subcloned in soft agar with an efficiency of 10–15%. Some of these S2 subclones have been maintained as principally haploid cultures for as long as 8 months and have a normal doubling time of 24 h. Thus, cultures of near-haploid P1-55 cells can be maintained indefinitely.

Work on haploid frog embyos indicated that the haploid cells were smaller than their diploid counterparts [11]. To determine whether this is also true of this cell line, populations of near-haploid and diploid subclones were mixed and the smaller cells in this population were sorted by light scattering. Figure 4 shows that the vast majority of small cells have a near-haploid DNA



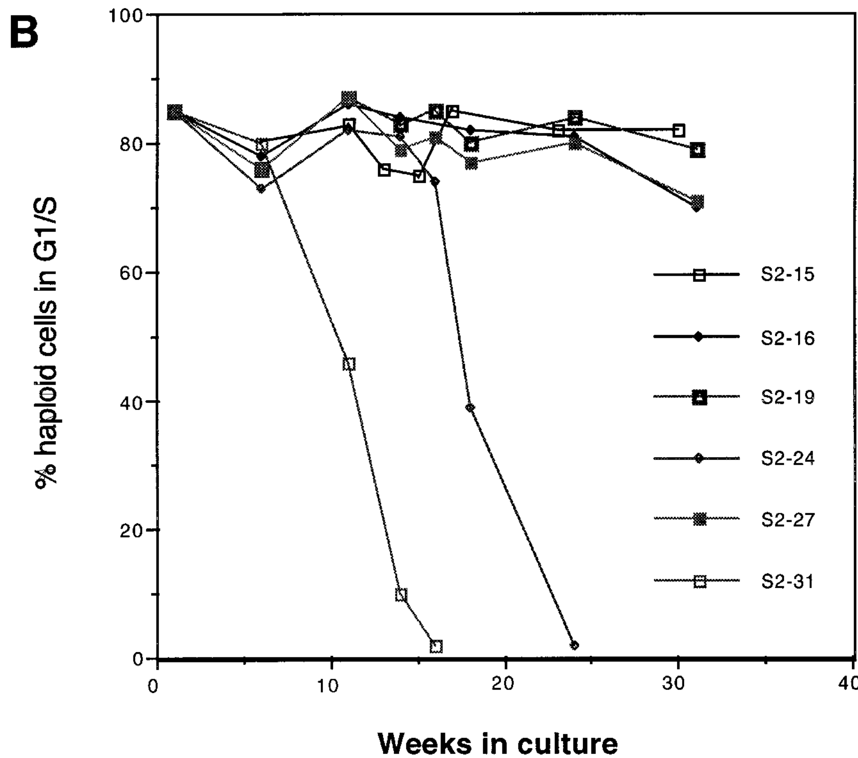




**FIG. 3.** DNA content change of near-haploid cell lines as a function of time in culture. (A) Subclones P1-32 and P1-55 of the KBM-7 cell line were isolated by cloning in soft agar. P1-55-S1 was isolated by subcloning P1-55. The DNA content of these cell lines was determined by FACS analysis at the indicated numbers of weeks in culture by the methods described under Materials and Methods. The percentage of haploid cells in G0/G1 and S was estimated either by using the MODFIT program (Verity Software House) or by direct peak area measurement. The percentage of haploid cells in G2 and M at any given time point could not be precisely determined because of the overlapping diploid cell peak in G0/G1. Twenty-one independent measurements of populations of haploid cells with no $4n$ peaks (no detectable diploid cells) indicate that the percentage of haploid cells in G1 + S at any time in culture ranges from 73 to 94% with a mean of 83%. The zero time point for the cloned cells begins with the day the cells were isolated from soft agar. At this time, too few cells existed to analyze by FACS. Assuming the cells were purely haploid at that time, approximately 83% would be in G1 + S. The dashed lines indicate this assumption for the zero time point. Since KBM-7 was not clonal, this assumption was not made. All other data points are actual measurements. The relative and absolute stability of each of the subclones remained consistent through multiple trials. Representative experiments are shown. (B) Stability of second-generation subclones (S2) of near-haploid P1-55 cells obtained after a second round of subcloning in soft agar was estimated for DNA content using flow cytometry.

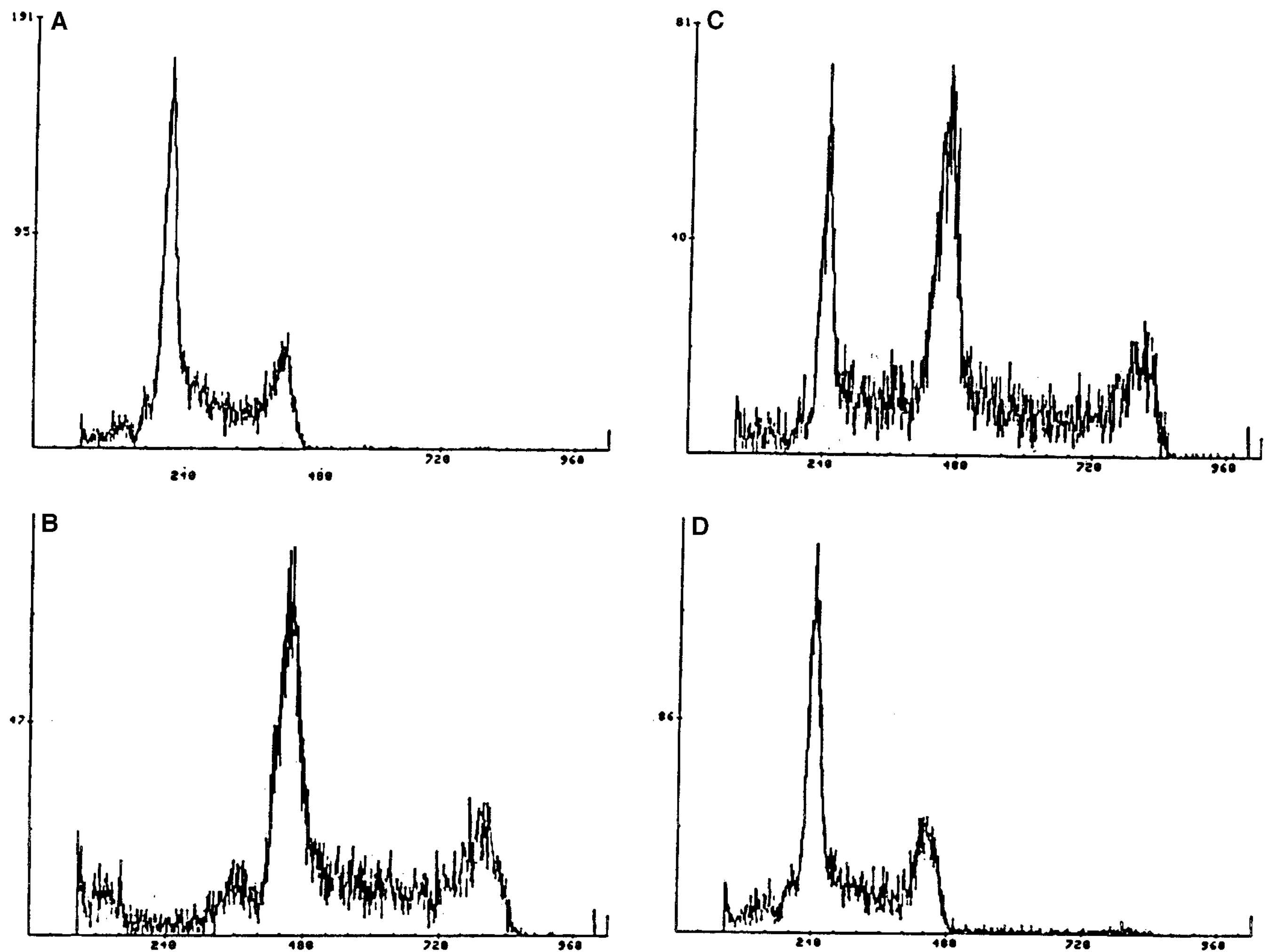



**FIG. 4.** Sorting of haploid cells based on size. A mixture (C) of haploid (A) and diploid (B) cells was sorted for the smallest cells using forward and side scatter analysis in flow cell sorter without any staining. Each histogram shows analysis of DNA content of a population of viable cells stained with Hoechst 33342. (A) DNA content of haploid clone S2-19. (B) DNA content of diploid clone S2-8. (C) DNA content of haploid S2-19 and diploid S2-8 cells mixed (#/# 1:1) right before size-sorting. (D) DNA content of smaller cells sorted out of the mixture of haploid and diploid cells (shown in C).

content. Thus, this technique can be used to isolate haploid cells from mixed ploidy populations.

## DISCUSSION

The existence of the P1-55 cell line demonstrates that full diploidy is not required for the maintenance of human cells in culture. In fact, the existence of this cell line demonstrates that every chromosome except perhaps chromosome 8 can exist as a monosome in culture. These cells are obviously not normal since they have the Philadelphia translocation in addition to the change in ploidy. However, they do have characteristics typical of many other mammalian cell lines such as a requirement for fetal calf serum for growth and the ability to maintain an exponential doubling time of approximately 12–30 h depending on the culture density (data not shown). From these observations, it can be inferred that if there are genes required in more than one copy for growth of human cells in culture, these must be a small percentage of the human genome.

The reasons for the maintenance of the disomy of chromosome 8 are unclear. It could be that there are genes on chromosome 8 that are required in two copies for the growth of the cells. Alternatively, there may be a gene on chromosome 8 that contributes to the establishment of the tumor or cell line (such as the *myc* gene) when maintained in multiple copies by the presence of the disomy. Yet another possibility is that there are independent recessive cell lethal mutations on both

homologues of chromosome 8 that are mutually complemented by the presence of the corresponding wild-type alleles on the other copy of 8. This would be consistent with the finding that there is heterozygosity at chromosome 8 in this cell line. We favor one of the latter two hypotheses since previous analyses of near-haploid leukemias do not always show disomic chromosome 8 [12, 13].

The mechanism by which these cells became haploid is unclear. It is possibly the result of an abnormal karyokinesis induced by chemotherapeutic agents used to treat this patient. Alternatively, specific genetic alterations may have contributed to the haploidization event. In yeast, a gene has been described that has the phenotype of frequent mitotic haploidization [14]. Perhaps a similar gene is mutated in these cells.

Despite their unusual karyotype, haploid vertebrate cells are likely to be capable of exhibiting a wide range of cellular responses. Haploid fish and amphibians though not viable show a significant degree of morphological and cellular differentiation and in some cases can live for several weeks [15–18] Moreover, haploid-euploid mosaic chickens are not only viable, but show representation of haploid cell lineages in a variety of tissues [19]. Thus, haploid cells could serve as good models of their diploid counterparts for a variety of biological phenomena.

The ability to isolate recessive mutations at a relative high frequency from Chinese hamster ovary (CHO) cells has led to the suggestion that the CHO cell genome is functionally hemizygous [20]. However, analysis of electrophoretic shift variants of CHO is consistent with the near-diploid karyotype of this cell line [21, 22]. The high rate of recessive mutant isolation is more likely due to a high frequency of deletion and chromosome loss uncovering relatively rare single gene mutations [23–25]. The advantage of a truly haploid cell line such as P1-55 is that there would be no need for secondary events to occur, thus increasing the relative frequency of phenotypic expression of recessive traits. In addition, it is not known with any certainty what percentage of the CHO cell genome is available for high-frequency isolation of recessive mutants, whereas in P1-55 virtually all loci not on chromosome 8 should be inactivated with single hit kinetics.

Thus, it should prove possible to use the P1-55 cell line and its derivatives to facilitate the application of somatic cell genetics to the study of mammalian cell biology. For instance, insertional mutagenesis of these cells by high-titer retroviruses should inactivate genes with a single hit, leaving the inactivated gene tagged with retroviral DNA and thereby facilitating the recovery of the affected loci [26]. Furthermore, use of vectors designed for homologous recombination in somatic cells has proved to be very useful for the analysis of diploid cultured cells [27–29]. Homologous recombination with these near-haploid cells would allow for the immediate expression of the phenotype of inactivated genes without the necessity of knocking out two alleles or passaging the gene knockout through chimeric animals [30]. The fact that these cells have a tendency to increase in ploidy with time in culture should not undermine the effectiveness of these genetic strategies since any genetic alteration that occurs in a haploid cell will presumably be duplicated and remain homozygous as diploidization occurs. Moreover, we have been able to reisolate near-haploid cells from mixed ploidy populations by both size sorting and subcloning. Therefore, these near-haploid cells provide several advantages over diploid ones for somatic cell genetics.

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