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Creation of monosomic derivatives of human cultured cell lines

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ABSTRACT Monosomic mammalian cell lines would be ideal for studying gene dosage effects, including gene imprinting, and for systematic isolation of recessive somatic mutants parallel to the invaluable mutants derived from haploid yeast. But autosomal monosomies are lethal in early development; although monosomies appear in tumors, deriving cell lines from these tumors is difficult and cannot provide several syngenic lines. We have developed a strategy for generating stable monosomic human cells, based on random autosomal integration of the *gpt* plasmid, partial inhibition of DNA topoisomerase II during mitosis to promote chromatid nondisjunction, and selection against retention of *gpt*. These are likely to be valuable as a source of otherwise inaccessible mutants. The strategy can also be used to generate partial mammalian monosomies, which are desirable as a source of information on recessive genes and gene imprinting.

Monosomic mammalian cell lines are experimentally desirable for several reasons. Mouse embryos have been produced that are monosomic for virtually every chromosome, but they rapidly perish in the early postimplantation period, between early and late blastocyste stage (1). In one case, monosomy 19, loss occurred both in outbred and in inbred mouse strains; hence, early mortality in monosomy is caused by dosage or imprinting effects rather then by haploid expression of lethal genes (2). This result may also be true in humans. Therefore, disomic and derived syngenic monosomic human cell lines would be needed for studying gene dosage and imprinting effects in humans, and partial mouse embryonic monosomies would be useful if they are less immediately lethal.

Monosomic human lines are also desirable for isolating human autosomal recessive mutants. Many useful cell cycle and DNA repair mutants been obtained from haploid yeast, including temperature-sensitive conditional lethals. Mammalian systems appear more complex, but mammalian genetics is less easy, because only viable germ-line mutants, or dominant or X-linked somatic cell mutants, are normally detectable. Fortuitously, some transformed rodent cells produce recessive autosomal mutants; notably the Chinese hamster line CHO-K1. This cell line has a somewhat rearranged karyotype (3), and a substantial fraction of the genome is either structurally monosomic or functionally hemizygous, the latter probably as a result of aberrant imprinting (4). Many mutants of CHO and similar lines have been created, including DNA repair and cell cycle mutants, and cells with genome instability. But these are unlikely to be fully representative of the range of possible human mutants. Some of the CHO genome remains functionally diploid; worse, transformed rodent cells differ in important ways from human cells. They have very different DNA excision repair strategies (5) and DNA synthetic activities after damage (6,7) and have leaky cell cycle checkpoints (8–10). Human tumor biopsies often contain cells with monosomies, from which recessive mutants could in principle be isolated (11). Monosomy of any one chromosome, except possibly 2 and 4, appears nonlethal in tumors (12). But establishing cultured cell lines from tumors is time consuming and could not provide different syngenic monosomies. Instead, we have developed a general strategy to generate viable and stable monosomies, even including chromosome 4, in transformed or immortal human lines.

The strategy involves perturbation of mitotic DNA topoisomerase II, which decatenates the DNA of sister chromatids, and is essential for anaphase chromatid disjunction in yeast (13,14). High levels of chemical inhibitors of topoisomerase II similarly completely block disjunction in mammalian cells (15, 16). Because ts*top2* yeast show increased spontaneous chromosome loss even at the permissive temperature (17), we argued that partial inhibition of human mitotic topoisomerase II should prevent disjunction of one or a few chromosomes, producing monosomic and trisomic daughters. We therefore combined topoisomerase II partial inhibition with a dominant-positive/negative genetic selection system for isolating the monosomics.

PROCEDURES

Cell Culture, Transfection, and Synchrony. Cells were grown as monolayers in Eagle's minimal essential culture medium. T4C15 is a chicken/CHO hybrid cell, containing a nonessential chicken marker chromosome that bears a functional hypoxanthine phosphoribosyltransferase (HPRT) gene (18). EJ30 is a bladder carcinoma line with quasi-diploid karyotype and excellent cloning properties (19). MSU1.1 is a v-*myc*-immortalized but nontransformed human cell line with minimal chromosomal aberrations (20). An HPRT$^-$ mutant was produced from MSU1.1 cells by ethyl methanesulfonate (EMS) mutagenesis. A spontaneous EJ30 HPRT$^-$ mutant, EJ30^{tgr}, was a gift from David Hatton (CRC, Cambridge, U.K.). The stability of each mutation was tested by incubating 10^6 cells with medium containing HAT (hypoxanthine/aminopterin/thymidine); no survivors arose from either of the HPRT$^-$ lines. HPRT$^-$ cells were transfected by electroporation with pPMH16 (containing the *gpt* and *neo*^{r} genes) and selected with HAT medium. EJ30 cells and their transfected

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Abbreviations: HPRT, hypoxanthine phosphoribosyltransferase; HAT, hypoxanthine/aminopterin/thymidine; FISH, fluorescent *in situ* hybridization; CGH, comparative genomic hybridizations.
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derivatives and T4C15 were synchronized in metaphase with a thymidine/nitrous oxide protocol (21). Nitrous oxide is toxic to MSU1.1, so these, and their transfected derivatives, were synchronized by release from contact inhibition in G_1, followed by shake-off of mitotic cells 12 h later.

Topoisomerase II Perturbation. We used the specific topoisomerase II inhibitors etoposide, which produces DNA strand breaks, and ICRF-193 which produces no collateral DNA damage (16, 22). Cells were presynchronized in mitosis to avoid complications caused by the G_2 topoisomerase II cycle control checkpoint (23). EJ30-derived transfectant metaphase-arrested cells were treated after release with 5 μM etoposide for 2 h. This dose yields about 60% survival and causes chromosomes to lag at anaphase in about 30% of the cells. MSU1.1-derived transfectants were treated with etoposide or ICRF-193 (350 nM) for 2 h while passing through mitosis in the absence of microtubule antagonists.

Selection of Monosomics. After release from arrest, treatment with topoisomerase II inhibitors to promote nondisjunction, and 2-day recovery, EJ30 and MSU1.1 transfectants were selected for 6-thioguanine resistance. This procedure yields segregants that have lost the *gpt* gene, through nondisjunction of the autosome (or loss of the autosomal region) containing the pPMH16 plasmid, and are therefore monosomic for the other chromosome of that pair, or a region thereof. 6-Thioguanine-resistant colonies were cloned, propagated, and analyzed.

Karyotyping. Harvesting and chromosome preparation for cytogenetic analysis followed standard procedures. Karyotyping was performed with IKAROS-system (Metasystems, Altlussheim, Germany). The karyotypes of the parent cell lines were as follows: MSU-1.1 45, XY, −11, −12, −15, dup(1)(q11), +der(11), t(1;11)(p11;p15), del(12)(p11), +der(15), t(12;15)(q11;p11). This is the same as in the original description of these cells (19); EJ30^{tgr} 46, X, −Y, +der(11)t (11;20)(11pter(11q11::20q11(20qter), del(4)(q28q32), dup(7) (q32q35), der(8)(7q32(q35::8p12(8qter), del(9)(p13), der(10) (22qter(22q11.2::10p11 (10qter), t(15;18)(15qter(15q21::18p11 (18qter).

Fluorescent *in Situ* Hybridization (FISH). The FISH protocol followed that of Pinkel *et al.* (24). Probe DNA was labeled with biotin-16-dUTP via nick-translation. Hybridization was carried out in 50% formamide, 10% dextran sulfate, 1× standard saline citrate (SSC), 0.05 μg/μl biotinylated probe DNA, and 0.1 μg/μl salmon sperm DNA for 16–20 h at 37°C. Slides were viewed on a epifluorescence microscope (Zeiss Axiophot), and photographs were taken on Agfachrome 1000 RS 135 color slide film or by a cooled charged-coupled device (CCD) camera (Hamamatsu) that was controlled by ISIS software (Metasystems).

Fluorescent whole chromosome painting probes were obtained from Vysis (Stuttgart, Germany) (direct labeled) and Oncor (indirect labeled). Hybridization and detection was performed according the manufacturers' protocol. Comparative genome hybridization was performed as described by Kallioniemi *et al.* (25) with slight modifications. Genomic DNAs were labeled via nick translation with biotin-16-dUTP (Boehringer Mannheim) (test DNA) and digoxigenin-11-dUTP (Boehringer Mannheim) (reference DNA). For each hybridization, 400 ng of labeled test DNA, 400 ng reference DNA, and 50 μg C_ot^{-1} DNA were used. Comparative genomic hybridizations (CGH) were analyzed by using an Axiophot microscope (Zeiss) equipped with a cooled CCD camera (Hamamatsu). Image analysis and karyotyping was performed with an ISIS system (Metasystems).

***In Situ* PCR.** For the detection of residual intact *gpt* genes in the segregants the following primer pairs were used: gpt 1 (5′-ACA CAA GAC AGG CTT GCG AG-3′) and gpt 2 (5′-CGC TGG TTG TCG TGA TCG TA-3′). PCR conditions were as follows: denaturation at 92°C for 5 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min. After 40 cycles of amplification, the products underwent a final extension at 72°C for 8 min. As positive controls the transfectant cell lines and pPMH16 were used.

RESULTS

Creation of Monosomy by Partial Nondisjunction. As a first test of the hypothesis that perturbation of mitotic topoisomerase II should cause chromosome loss, hybrid T4C15 cells, containing a nonessential chicken marker chromosome (bearing the HPRT gene), in a HPRT$^-$ CHO background (18), were treated in mitosis with ICRF-193 and etoposide to produce partial topoisomerase II inhibition, and tested for loss of the chicken chromosome by the ability to resist 6-thioguanine which is metabolized by HPRT (26). Resistant colonies were produced, all of which had lost the chicken chromosome (data not shown).

For selection of human monosomics we used a system (Fig. 1) that employs the same well-characterized purine synthesis pathway (26). The endogenous, X-linked HPRT gene was first mutated to produce HPRT$^-$, 6-thioguanine-resistant human cells. These HPRT$^-$ mutants were then transfected with the plasmid pMH16, containing the bacterial *gpt* gene, which also allows for purine scavenging, and the *neo*^{r} gene. HPRT$^-$*gpt*$^+$ cells were selected for their ability to survive in HAT medium,

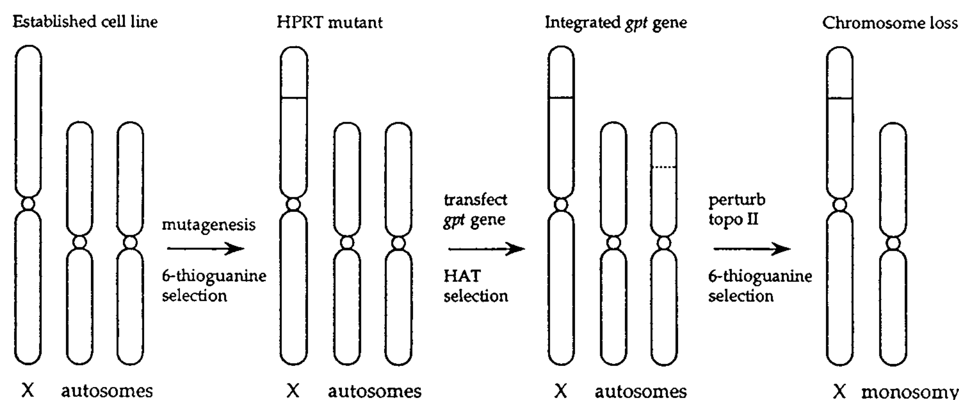

FIG. 1. Selection protocol for generating monosomic cell lines. An established near diploid cell line is mutagenized and selected with 6-thioguanine to isolate an HPRT mutant clone. The bacterial homologue of the HPRT gene (*gpt*) is then integrated into a single autosome by transfection and selection with medium containing HAT. Chromosome loss is induced by perturbing anaphase segregation with topoisomerase II inhibitors. Segregants that have lost the gpt marked autosome are isolated by selection with 6-thioguanine. Mutation is indicated by a horizontal line, and the integration site is indicated by a discontinuous horizontal line.

which contains hypoxanthine as a purine source, aminopterin as an inhibitor of endogenous nucleotide synthesis, and thymidine as a pyrimidine source. Crucially, stable transfection of plasmids such as *gpt* into human cells involves integration at low copy number and at random, single sites (27); such low-copy integration can be achieved by electroporation even in mouse cells (28). HPRT^{-}*gpt*$^{+}$ cells were then arrested at metaphase. On release from arrest, they were treated with low concentrations of etoposide (5 μM) or ICRF-193 (500 ng/ml) to promote nondisjunction. They were subsequently selected for 6-thioguanine resistance. This process yielded segregants that have lost the *gpt* gene through nondisjunction of the autosome (or loss of the autosomal region) containing the embedded plasmid and are therefore monosomic for the other chromosome of that pair or region thereof. This protocol has been found to produce monosomics from two human cell lines tested: EJ30, a bladder carcinoma line that we chose for its quasi-diploid karyotype and excellent cloning properties, and MSU1.1, an immortalized line with minimal chromosomal aberrations.

Cytogenetic Analysis. We have confirmed, by G-banding, CGH, and chromosome painting, the existence of partial trisomy 1 in MSU1.1 and partial chromosome losses in EJ30, and verified additional aberrations in its HPRT^{-}-derived line, EJ30^{tgr}, as trisomy 11p and trisomy 20q, dup (7)(q32–35) and loss of the Y chromosome.

Chromosomal integration sites of the *gpt* gene in HPRT^{-}*gpt*$^{+}$ cells have been identified by FISH. Initially we completely characterized one transfectant derived from each of EJ30 and MSU1.1. The EJ30-derived HPRT^{-}*gp*t^{+} transfectant GHR3 has a prominent integration site in the centromeric region of a submetacentric chromosome as shown after FISH (Fig. 2*a*). The labeled chromosome corresponds to a derivative human chromosome 11 as seen after chromosome painting whereby the original short arm was deleted and replaced by an element of unknown origin (Fig. 2*b*). Further proof was sought by creating hybrids from GHR3-derived minicells and a triploid Indian muntjac cell line, then selecting with G418 medium for *neo*^{r}. Such hybrids retain a small number of human chromosomes, including the chromosome carrying the *gpt* and *neo*^{r} marker genes. Human chromosomes can be easily distinguished from the muntjac chromosomes due to the large difference in size. G-band and FISH analyses were again used to show that the hybrids made from GHR3 contained the human chromosome 11 (data not shown).

The MSU1.1-derived transfectant T34 was similarly characterized to show that the marker gene resides in chromosomes 1, 4, and 10 (Fig. 2*c*) and CGH illustrates that trisomy 1q is the only aneuploidy (Fig. 2*d*).

GHR3 and T34 cells were treated with topoisomerase II inhibitors in mitosis so as to generate potential segregants, then grown in 6-thioguanine medium and surviving clones isolated after 10–14 days. 6-Thioguanine-resistant colonies

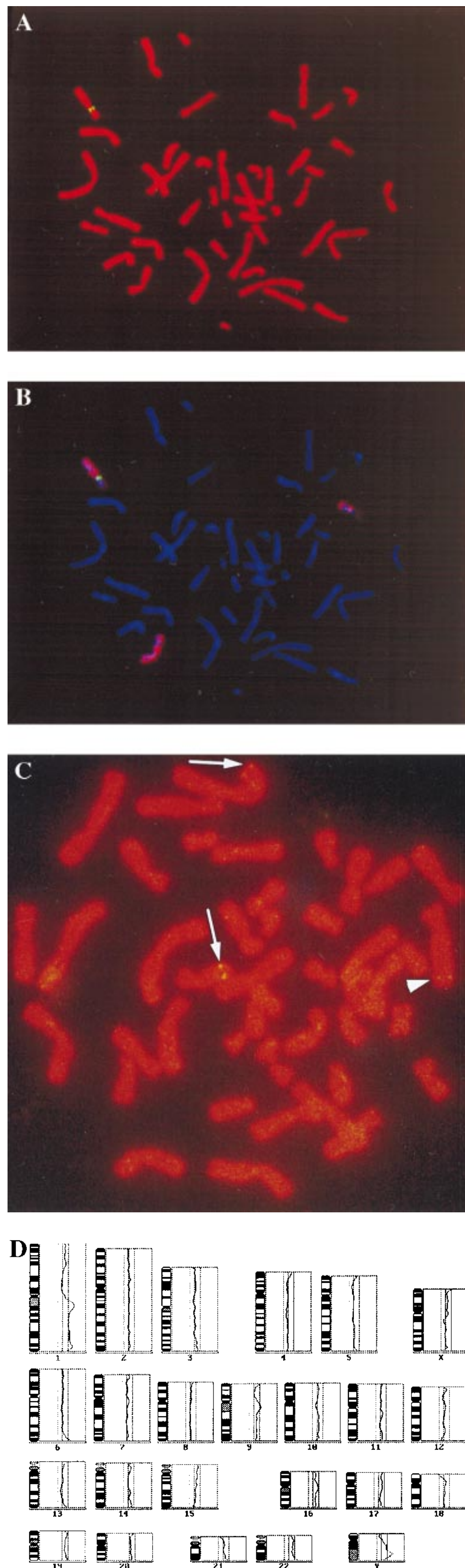

FIG. 2. Verification of the chromosome location of the *gpt* gene in the GHR3 clone, a transfectant of EJ30, and in T34, a MSU1.1 transfectant cell line, and analysis of the T34 karyotype by comparative genomic hybridization. (*A*) *In situ* hybridization of the transfectant cell line GHR3 by using a pPMH16 generated probe for *gpt*, detected with avidin-FITC, gives a clear signal in the centromeric region of one C-group chromosome. The chromosomes are counterstained by propidium iodide. (*B*) The same metaphase spread as in A after reprobing with a chromosome painting probe for chromosome 11. The chromosome bearing the marker pPMH16 is identified as a derivative chromosome 11. In addition there is a normal chromosome 11 and a derivative 11p bearing material of chromosome 20q. (*C*) Metaphase spread of T34 after FISH with the biotinylated probe pPMH16. Signals are seen in chromosome 1 (arrow), chromosome 4 (arrowhead), and chromosome 10 (arrow). (*D*) Average ratio profile (curve) from pter to qter (for each chromosome) obtained from CGH analysis of the MSU1.1-derived transfectant cell line T34. The baseline ratio value (1.0) and thresholds 0.80 (*Left*) and 1.25 (*Right*) are shown as vertical reference lines. Chromosome ideograms (inverted G-banding) are shown for approximate visual reference. Only the ratio value of chromosome 1q falls outside the normal range (partial trisomy 1q). Heterochromatic regions, centromeres, and telomeres are excluded from evaluation.

arose at a frequency of about 1 in 10^4 or 2 in 10^4 surviving mitotic cells from GHR3 or T34, respectively.

6-Thioguanine-resistant clones derived from T34 and GHR3 were analyzed first by preparing stained chromosome spreads to determine chromosome number (25–50 spreads scored; Table 1). Clones derived from the EJ30 transfectant, like their parent, typically contained a small (around 8%) proportion of hyperploid (near-triploid, near-tetraploid, etc.) cells that were not included in the chromosome counts; the remaining cells contained 42–46 chromosomes. T34 putative segregants had a range of chromosome numbers from 42 to 44.

Because almost all of the GHR3- and T34-derived segregants had a lower chromosome number than the parent lines, their karyotypes were analyzed in detail. Initially we applied both G-banding and comparative genomic hybridization analysis and confirmed the aberrant chromosomes by chromosome painting and the integration site of the plasmid vector by FISH analysis. First by using G-band and CGH analysis, and later by confirmatory chromosome painting, we found that one of the segregants derived from T34, VP34-6, is monosomic for the whole of chromosome 4 (Fig. 3*a*), whereas two, VP34-2 and VP34-7, show a deletion of the distal part of 4q including the integration site of the plasmid (Table 1). FISH also showed that these segregants no longer have the *gpt* signal in 4q or elsewhere characteristic of the parent line T34. Remarkably, line VP34-3 still has the original chromosome set but has lost the marker, as confirmed by PCR. Thus, these data are consistent with our understanding of the selection system; the *gpt* marker has been lost in all cases, caused by partial or complete monosomy of the relevant chromosome and/or selective loss of the integrated plasmids.

For GHR3, the segregants also have some substantial monosomies, though not in all cases for the reasons we expected. VP9 is monosomic for chromosome 8 (Fig. 3*b*), and VP10 is monosomic for chromosome 12p (isochromosome 12q/trisomy 12q), and VP22 is monosomic for chromosome 4 (Fig. 3*c*). Clearly in these cases, however, the monosomic regions do not correspond to the position of the marker in GHR3 (chromosome 11). Despite this, no *gpt* signal is detectable by FISH in these segregants. To investigate this finding further, genomic DNA from these cell lines was blotted onto nitrocellulose, then probed with [^{32}P]ATP-labeled pPMH16.

Table 1. Cytogenetic analysis of MSU1.1 and EJ30^{tgr} cell lines, their *gpt* transfectants, and the monosomic and partial monosomic segregants

Parent	Transfectant	Segregant	Mean chromosome number	Induced (partial) monosomies
MSU1.1			45	
	T34		45	
		VP34-6	43 (43–44)	Monosomy 4
				Partial monosomy
		VP34-7	45	4q33(qter)
				Partial monosomy
		VP34-2	44 (42–44)	4q3 (qter)
				Partial monosomy
		VP34-5	44	6pter(q2)
		VP34-3	45	No deletion
EJ30^{tgr}			46 (44–47)	
	GHR3		45 (42–47)	Monosomy 21
		VP22	43 (42–44)	Monosomy 4
		VP9	42–43	Monosomy 8
			(39–46)	Partial monosomy
				6 pter(q1)
				7q2(qter)
		VP7	43	Partial monosomy
				der 11p10(pter)
				3q10(qter)

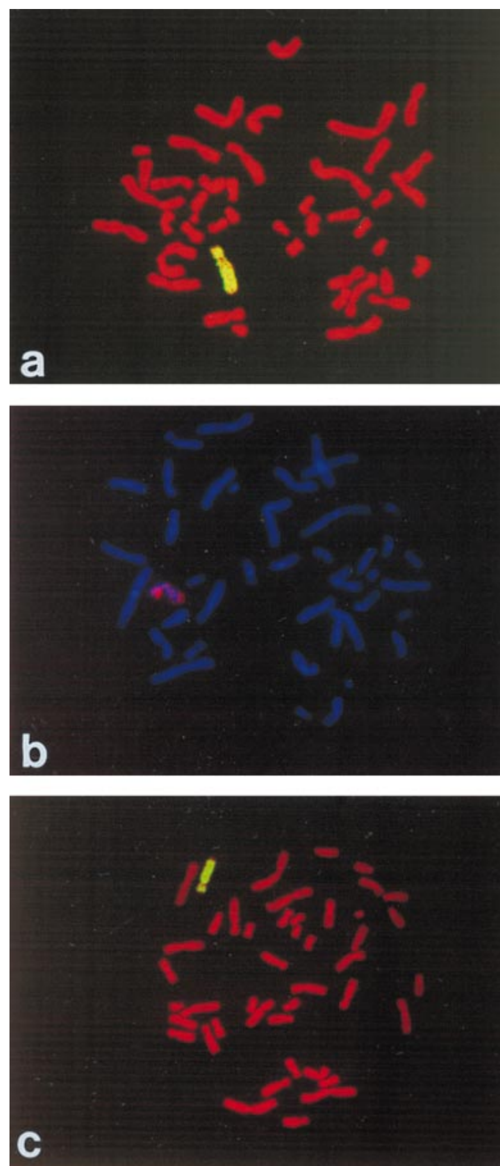

FIG. 3. Verification, by chromosome painting, of monosomies in MSU1.1 and EJ30-derived cell lines. (*a*) Monosomy 4. Metaphase spread of the MSU1.1-derived VP34-6 segregant cell line after chromosome painting with a chromosome 4 probe. (*b*) Monosomy 8. Metaphase spread of the EJ30-derived VP9 segregant cell line after chromosome painting with a chromosome 8 probe. (*c*) Monosomy 4. Metaphase spread of the EJ30-derived VP22 segregant cell line after chromosome painting with a chromosome 4 probe.

This result revealed that pPMH16 DNA still resides in the VP9 genome. It appears that VP9 has lost a proportion of the total plasmid that integrated into the GHR3 genome.

Monosomy 4 in VP22 and monosomy 8 in VP9 could have resulted from random nondisjunction events; loss may not have been selected for because the *gpt* integration site in the parental line (GHR3) is in chromosome 11. Alternatively, it is possible that chromosomes 4 and 8 in GHR3 have a site in which a small number of *gpt* sequences exist that are not detectable by FISH. Interestingly, VP7 shows a 3/11 translocation in which the breakpoint obviously coincides with the plasmid integration site in chromosome 11.

Each cell line was also karyotyped at late passage (after several months in culture); there was no evidence during this time of endoreduplication to reinstate disomy. Table 1 summarizes the information about GHR3, T34, and their derivative lines. In addition, other transfectants and segregants are

described that have not yet been fully characterized. All of the monosomics grow well in culture and have plating efficiencies and generation times similar to their parent cell lines (data not shown).

DISCUSSION

Monosomic human cells have not been established previously by design in culture. Established human monosomic cell lines would be an ideal tool to studying gene dosage effects at the RNA level, or (as has been done for trisomies) at the protein level by two-dimensional protein electrophoresis (29, 30).

In addition these cell lines would make excellent progenitors for use in mutant screens, generating valuable human mutants that would otherwise be inaccessible by chemical mutagenesis; or by the use of knockout vectors (31), which produces valuable information only about the role of previously identified genes. Even the recent development of homozygous knockout antisense vectors, as a technique for generating recessive mutants in unknown genes (32), is not applicable to the generation of desirable temperature-sensitive mutants. In addition, imprinting of genes that are not essential for somatic cell growth may also be detectable in monosomic clones.

We have developed a straightforward technique for generating human somatic monosomic clones. Monosomy for chromosome 4 or 8 is stable at late passage and is a nonlethal event in the cell lines we have used; no obvious deleterious effects such as decreased plating efficiency or increased generation time have been observed, even with such a large chromosome as 4 monosomic. Tolerance of monosomies of smaller chromosomes has been reported for EJ30 (19); whether these cells can tolerate monosomy of even larger autosomes is unknown at this time. Partial monosomy is seen quite often in the segregants. In the case of the lines produced by etoposide treatment, double-strand breaks and subsequent loss of distal regions could account for this. ICRF-193 could have induced partial monosomy if chromosome breakage occurred during anaphase because of segregation blockage, or as a consequence of its complex actions on mitotic cores during mitosis (33).

Continuing work is focused on generating other monosomic clones, and on isolating cell cycle and DNA repair deficient mutants from the existing monosomics.

Recently, a strategy has been developed (34) for creating substantial deletions or rearrangements in the genomes of mouse embryo stem cells, with the aim of uncovering recessive mutant genes. Some 10% of the DNA of mouse chromosome 11 has been converted, through a series of recombinations, to a set of stable partial monosomies, each rather small. We would point out that the technique described here could easily be used on mouse stem cells to create more extensive partial monosomies with less effort.

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1. Gropp, A. (1982) *Virchows Arch.* **395,** 117–131.
2. Magnuson, T., Smith, M. A. & Epstein, C. J. (1982) *J. Embryol. Exp. Morphol.* **69,** 223–236.
3. Deaven, L. L. & Peterson, D. F (1973) *Chromosoma* **41,** 129–144.
4. Siciliano, M. J., Stallings, R. L. & Adair, G. M. (1985) in *Molecular Cell Genetics*, ed. Gottesman, M. M. (Wiley, New York), pp. 95–135.
5. Downes, C. S., Ryan, A. J. & Johnson, R. T. (1993) *BioEssays* **15,** 209–216.
6. Ryan, A. J., Squires, S., Strutt, H. L., Evans, A. L. & Johnson, R. T. (1994) *Carcinogenesis* **15,** 823–828.
7. Johnson, R. T., Downes, C. S., Godfrey, D. B., Hatton, D. H., Ryan, A. J. & Squires, S. (1994) in *The Legacy of Cell Fusion*, ed. Gordon, S. (Oxford Univ. Press, New York), pp. 50–67.
8. Downes, C. S., Musk, S. R. R., Watson, J. V. & Johnson, R. T. (1990) *J. Cell Biol.* **110,** 1855–1859.
9. Kung, A. C., Sherwood, S. W. & Schimke, R. T. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 9553–9557.
10. Steinman, K . E., Belinski, G. S., Lee, D. & Schlegel, R. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6843–6847.
11. Busch, D. B. (1989) *Mutagenesis* **4,** 486.
12. Human Gene Mapping 10 (1989) *Cytogenet. Cell Genet.* **51,** 1–1148.
13. di Nardo, S., Voelkel, K. & Sternglanz, R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2616–2620.
14. Uemura, T. H., Ohkura, Y., Adadi, R., Mormo, R., Shiozadi, R. & Yanagida, M. (1987) *Cell* **50,** 917–925.
15. Downes, C. S., Mullinger, A. M. & Johnson, R. T. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 2616–2620.
16. Clarke, D. J., Johnson, R. T. & Downes, C. S. (1993) *J. Cell Sci.* **105,** 563–569.
17. Hartwell, L. H. & Smith, D. (1985) *Genetics* **110,** 381–395.
18. Burg, K. & Raskó, I. (1982) *Mutat. Res.* **97,** 176.
19. Kovnat, A., Buick, R. N., Choo, B., de Harven, E., Kopelyan, I., Trent, J. M. & Tannock, I. F. (1988) *Cancer Res.* **48,** 4993–5000.
20. Morgan, T. L., Yang, D., Fry, D. G., Hurlin, P. J., Kohler, S. K., Maher, V. M. & McCormick, J. J. (1991) *Exp. Cell Res.* **197,** 125–136.
21. Johnson, R. T., Downes, C. S. & Meyn, R. E. (1994) in *The Cell Cycle: A Practical Approach*, ed. Fantes, P. & Brooks, R. (IRL, Oxford), pp. 1–28.
22. Roca, J., Ishida, R., Berger, J. M., Andoh, T. & Wang, J. C. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1781–1785.
23. Downes, C. S., Clarke, C. J., Mullinger, A. M., Giménez-Abián, J. F., Creighton, A. R . & Johnson, R. T. (1994) *Nature (London)* **372,** 467–470.
24. Pinkel, D., Straume, T. & Gray, J. W. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2934–2938.
25. Kallioniemi, O. P., Kallioniemi, A., Piper, J., Isola, J., Waldman, F. M., Gray, J. W. & Pinkel, D. (1994) *Genes Chromosomes Cancer* **10,** 231–243.
26. Szybalska, E. H. & Szybalski, W. (1962) *Proc. Natl. Acad. Sci. USA* **48,** 2026–2034.
27. Hoeijmakers, J. H. J., Odijk, H. & Westerveld, A. (1987) *Exp. Cell Res.* **169,** 111–119.
28. Boggs, S. S., Gregg, R. G., Borenstein, N. & Smithies, O. (1986) *Exp. Hematol.* **14,** 988–994.
29. Klose, J., Zeindl, E. & Sperling, K. (1982) *Clin. Chem.* **28,** 987–992 .
30. Klose, J. & Putz, B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3753–3757.
31. Itzhaki, J. E., Gilbert, C. S. & Porter, A. C. G. (1997) *Nat. Genet.* **15,** 258–265.
32. Li, L. & Cohen, S. N. (1996) *Cell* **85,** 319–329.
33. Giménez-Abián, J. F., Clarke, D. J., Mullinger, A. M., Downes, C. S. & Johnson, R. T. (1995) *J. Cell Biol.* **131,** 7–17.
34. Ramirez-Solis, R., Liu, P. & Bradley, A. (1995) *Nature (London)* **378,** 720–724.