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Chromosome-Mediated Gene Transfer of Multidrug Resistance

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Multidrug resistance can be transferred from drug-resistant LZ Chinese hamster cells to drug-susceptible mouse LTA cells by chromosome-mediated gene transfer. Analysis of genomic DNA demonstrated the transfer of multiple copies of a DNA domain which is amplified in the donor multidrug-resistant cells. The transfer of 10 to 15 copies of the Chinese hamster gene was sufficient to produce a multidrug-resistant phenotype. Chromosome transferents exhibited overexpression of an mRNA of approximately 5 kilobases which has previously been demonstrated to be encoded by the amplified DNA domain of the donor LZ cells. Phenotypic analysis of individual clones selected in adriamycin showed the resistance to be pleiotropic. All clones tested demonstrated similar levels of cross-resistance to the drugs daunorubicin and colchicine. These results indicate that the DNA sequences transferred confer the complete multidrug-resistant phenotype on recipient cells and suggest that multidrug resistance is due to overexpression of the protein encoded by the 5-kilobase mRNA.

The mechanisms by which tumor cells acquire resistance to cytotoxic drugs are of primary importance in chemotherapy, as the failure of chemotherapeutic treatment is most probably caused by an outgrowth of drug-resistant cells (22). This phenomenon, inherently difficult to investigate in vivo, has been studied intensely in vitro with cultured rodent and human cell lines. Stepwise selection of cell lines with a single cytotoxic drug often leads to cross-resistance to a wide variety of such agents. This phenomenon has been termed multidrug resistance.

Gene amplification has long been suspected as one means by which cultured cells develop drug resistance (21). The presence of double minute chromosomes and homogeneously staining regions in multidrug-resistant human and rodent cells is consistent with this mechanism (1, 8, 11). More recently, the development of in-gel renaturation techniques by Roninson (19) has allowed the direct visualization of amplified genomic DNA. Using this approach we have previously shown that LZ, a highly resistant hamster cell line selected with adriamycin (9), and C5, a highly resistant hamster cell line selected with colchicine (12), have amplified a common portion of their genome (20). A 1.1-kilobase (kb) *Bam*HI fragment was cloned from this amplified unit (7) and allowed us to initiate the isolation and characterization of 130 kb of contiguous DNA commonly amplified in C5 and LZ (7). Analysis of genomic DNA showed that this cloned domain contains a gene approximately 75 kb in size which is likely to be a member of a small family of related genes. RNA hybridization studies have shown that this gene(s) encodes a 5-kb cellular RNA transcript whose level of expression correlates with the level of drug resistance in several multidrug-resistant hamster cell lines. The mouse and human homologs of this gene(s) have also been found amplified in the multidrug-resistant mouse L1210 DN transplantable tumor cell line (6) and in multidrug-resistant derivatives of the human KB carcinoma cell line (5, 20a).

Gene transfer experiments should be decisive in determining whether these amplified DNA sequences are biologically active in establishing multidrug resistance. Debenham et al. (3) and Robertson et al. (18) have reported such experiments with DNA-mediated gene transfer. However, direct evidence for the transfer of donor-cell DNA sequences to recipient cells was not provided in these experiments. In our initial attempts to perform similar DNA-mediated transfer experiments, we found that the frequency of apparent multidrug-resistant transfectants was very low, approximately 1 drug-resistant colony per 10^7 cells. Furthermore, when the DNA of apparent transfectants was analyzed, these resistant colonies were found to result from amplification of endogenous multidrug resistance sequences. Analogous observations have been made by other groups (P. Gros and C. Stanners, unpublished data; D. Shen and M. M. Gottesman, unpublished data).

A number of factors are likely to contribute significantly to the relatively low efficiency of DNA transfer for multidrug resistance. First, the transfer of multiple copies of a genomic DNA sequence to a single cell by DNA-mediated gene transfer is difficult. Second, the transcription unit within the amplified domain is large (≥75 kb), and direct transfer of DNA of this size is technically difficult. This concern is borne out by the experiences of Shen and Gottesman who observed that successful transfer of human multidrug-resistant DNA sequences to mouse cells correlated with efforts to minimize shearing of donor DNA (unpublished data). To achieve higher efficiencies of gene transfer, we turned to the technique of chromosome-mediated gene transfer. This technique allows the efficient transfer of contiguous DNA segments of 1,000 kb or more, thereby offering major advantages for the transfer of large amplified DNA domains such as the multidrug resistance gene (14, 15; D. E. Housman and D. L. Nelson, *in* R. Kucherlapati, ed., *Gene Transfer*, in press). Results presented in this paper show that the multidrug-resistant phenotype of LZ cells can be transferred to drug-susceptible LTA mouse cells by chromosome-mediated gene transfer. In several independent multidrug-resistant clones which were analyzed, the transfer of resistance was linked to the transfer of 10 to 15 copies of the hamster gene onto the mouse genomic background. Transfer of the amplified hamster gene coincides in resistant clones with a 10- to 20-fold increase in the level of transcription of the 5-kb mRNA. These results provide strong support for the

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view that multidrug resistance is the direct result of high levels of expression of a gene encoded by the genomic domain amplified in LZ.

MATERIALS AND METHODS

Cell culture. Chinese hamster V79 cells and mouse LTA cells were grown in minimum essential medium (GIBCO Laboratories, Grand Island, N.Y.) lacking nucleotides and supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin, and streptomycin. The multidrug-resistant hamster cells 77A and LZ were grown in the same medium supplemented with adriamycin (Adria Laboratories) at 0.1 and 8 μg/ml, respectively. Chromosome transferents TC1, TK2, TC2, and TI were routinely passaged in the same basal medium supplemented with adriamycin at 0.1 μg/ml. All cells were grown on plastic surfaces at 37°C in an atmosphere containing 5% CO_2.

Chromosome-mediated gene transfer. Chromosome-mediated gene transfer was performed as described by Housman et al. (in press). Confluent cultures (50% confluency) of LZ cells (approximately 10^8 cells) were exposed to colchicine for 16 h at a concentration of 10 μg/ml. The high concentration of colchicine was necessary to arrest a significant fraction of the cell population in mitosis owing to the cross-resistance of LZ cells to colchicine (24). Mitotic cells (4.3×10^7 cells) were harvested by strong agitation of the tissue culture flasks and were lysed in an ice-cold solution of 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.1)–3 mM $CaCl_2$ (15 min at 0°C), and were then mechanically disrupted by repeated passages through a 22-gauge needle. Chromosomes were recovered by serial centrifugations and suspended in 7.0 ml of transfection buffer (20 mM HEPES, 140 mM NaCl, 5 mM KCl, 5.5 mM glucose, 1 mM Na_2HPO_4, final pH 7.10). A chromosome-calcium phosphate precipitate was allowed to form for 20 min at 20°C after the addition of $CaCl_2$ to a final concentration of 125 mM. Recipient LTA cells were plated at a density of 3×10^5 cells per 60-mm dish 16 h before transfection. A total of 5×10^6 LTA cells (14 dishes) were exposed for 20 min at 20°C to the chromosome-calcium phosphate precipitate. Fresh medium (5 ml) was then added to each plate, and cells were placed at 37°C. After 4 h, cells were shocked with dimethyl sulfoxide (10%), washed once, fed with fresh medium, and further incubated at 37°C. Forty-eight hours later, cells were subcultured 1 to 6 into selective medium containing adriamycin at 0.1 or 0.3 μg/ml. Drug-resistant colonies, first observed 28 days later, were cloned 6 to 8 weeks after transfection and expanded in culture.

Drug-resistant phenotype. Five hundred cells from each of the cell lines were plated in 35-mm tissue culture dishes containing 3 ml of medium supplemented with increasing concentrations of adriamycin and daunorubicin (20, 50, 100, 300, and 500 ng/ml) and colchicine (20, 50, 100, 200, 500, 1,000, and 2,000 ng/ml). Cells were fed every 4 days to maintain a constant drug concentration in the medium. Sixteen days later, colonies were fixed for 3 h in 4% formaldehyde, stained with 0.1% methylene blue, and counted. Experiments were done in duplicate. Data are presented as relative plating efficiency expressed as the percentage of the respective control cell line grown without drug.

DNA hybridization studies. High-molecular-weight DNA was prepared from the various cell lines by standard proteinase K treatment and successive phenol and chloroform extractions (13). DNA samples were digested with restriction endonuclease *Bam*HI or *Eco*RI. Before the final electrophoresis, the DNA concentration of each sample was determined by fluoroscopy with a DNA-specific dye (4,6-diamidino-2-phenylindole).

Digestion products were separated by electrophoresis on 1% agarose gels containing 0.04 M Tris acetate (pH 8) and 0.002 M disodium EDTA. Separated DNA fragments were transferred to reusable hybridization membranes (Zetabind; BioRad Laboratories, Richmond, Calif.). Capillary transfer was done for 16 h in the presence of 10× SSPE (100 mM sodium phosphate [23] [pH 7.0], 1.8 M NaCl, 10 mM disodium EDTA). Electroblot transfer was done for 4 h at 40 V in 12 mM Tris (pH 7.6)–6 mM sodium acetate–0.3 mM disodium EDTA. The blots were hybridized for 48 h in the presence of 0.75 M NaCl, 75 mM sodium citrate, 5× concentrated Denhardt solution (0.02% bovine serum albumin, 0.02% Ficoll, 0.02% polyvinylpyrrolidone), 0.1% sodium dodecyl sulfate, and 10 μg of denatured salmon sperm DNA per ml with 10^7 cpm (<10 ng) of the indicated hybridization probe. Radiolabeled DNA probes were synthesized with the Klenow fragment of DNA polymerase I with random hexadeoxyribonucleotides (P-L Biochemicals, Inc., Milwaukee, Wis.) and [^{32}P]dCTP (4). Blots were washed to a final stringency of 15 mM NaCl–15 mM sodium citrate–0.1% sodium dodecyl sulfate at 65°C and exposed to Kodak XAR-5 films for 16 to 36 h at −80°C with an intensifying screen.

RNA hybridization studies. Total cellular RNA was extracted with guanidine hydrochloride by standard procedures (2). Total RNA (10 μg, except for LZ, 5 μg) was fractionated on denaturing (formaldehyde-containing) agarose gels and transferred to a hybridization membrane (Gene Screen Plus; New England Nuclear Corp., Boston, Mass.) by electroblotting in 25 mM sodium phosphate buffer (pH 7.0). RNA-DNA hybridization was carried out for 18 h at 62°C in the presence of 1 M NaCl, 1% sodium dodecyl sulfate, 10% dextran sulfate, 100 μg of denatured salmon sperm DNA per ml, and <10 ng of ^{32}P-labeled hybridization probe (5×10^6 cpm). Blots were washed to a final stringency of 30 mM NaCl–3mM sodium citrate–1% sodium dodecyl sulfate at 64°C, followed by autoradiography.

RESULTS

Chromosome-mediated gene transfer experiment. Control dishes containing selective media and the chromosome precipitate alone yielded no colonies, indicating that viable LZ cells were not present in the chromosome preparation. No colonies were observed when untreated LTA cells were grown in medium containing adriamycin at either 0.1 or 0.3 μg/ml for 6 weeks at 37°C. Mouse LTA cells exposed to the chromosome precipitate and placed in selective medium containing adriamycin at 0.3 μg/ml yielded no colonies after 6 weeks of observation. On the other hand, transfected LTA cells exposed to 0.1 μg of adriamycin per ml yielded 14 colonies. This corresponds to a transfer efficiency of 1 drug-resistant colony per 3×10^5 cells. Drug-resistant colonies were first detected 4 to 6 weeks after the beginning of selection. The clones were picked at 8 weeks and transferred into 24-well plates. Four independent clones were expanded in culture and further characterized.

Multidrug-resistant phenotype of chromosome transferents. Four independent adriamycin-resistant clones (TC1, TC2, TK1, TI) along with LZ and LTA control lines were tested

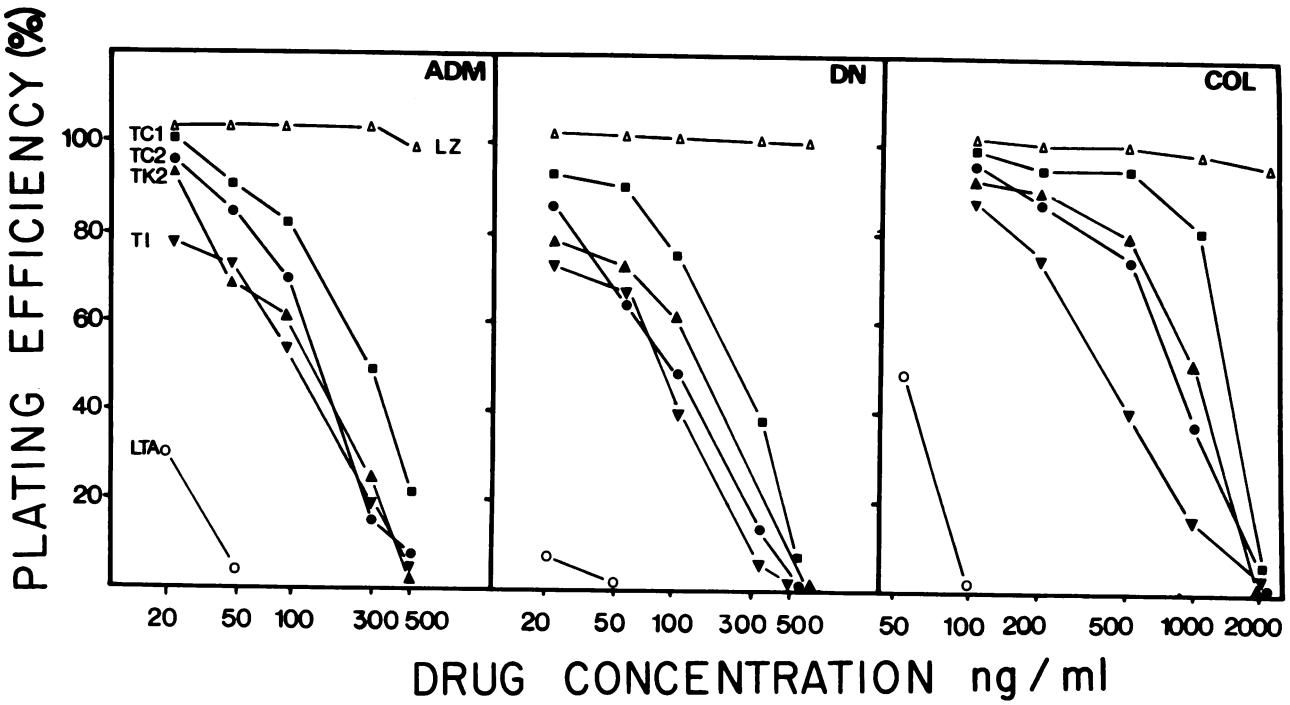

FIG. 1. Multidrug-resistant phenotype of chromosome transferents. Cells from chromosome transferents TC1 (■), TC2 (●), TK2 (▲), and TI (▼), multidrug-resistant LZ donor cells (△), and drug-sensitive susceptible LTA recipient cells (○) were plated at 5×10^2 cells per 35-mm dish in increasing concentrations of adriamycin (ADM), daunorubicin (DN), and colchicine (COL). At day 16, colonies were fixed, stained, and counted. Results are plotted as the percentage of surviving colonies compared with the respective controls plated without drugs versus the logarithm of the drug concentration.

for their level of cross-resistance to adriamycin, daunorubicin, and colchicine (Fig. 1). As expected, LZ donor cells showed a very high plating efficiency (nearly 100%) at the highest concentration of the three drugs tested, while LTA cells showed extremely poor plating efficiencies at drug concentrations above 20 ng/ml. All four chromosome transferents showed a very similar dose response to adriamycin. These transferents were all 10- to 20-fold more resistant to this drug than LTA cells. The multidrug-resistant phenotype of the four independently isolated clones was also quite similar. All four clones were approximately 10- to 20-fold more resistant to colchicine and daunorubicin than LTA cells.

The drug-resistant phenotype appears to be stable in these clones. After passage in culture without drug selection for several weeks, all four clones maintained a very similar pattern of drug resistance.

Analysis of genomic DNA. Genomic DNA from TC1, TC2, TK2, and TI as well as V79, 77A (Fig. 2, upper section), and LZ (Fig. 2, middle section) was analyzed for the presence of hamster-specific sequences by hybridization to DNA probes isolated from the amplified DNA domain of LZ cells. DNA (10 μg) digested with *Eco*RI (Fig. 2B, b; D, d; E) or *Bam*HI (Fig. 2A, a; C, c) was electrophoresed and transferred to hybridization membranes. The blots were probed with four repeat-free DNA subclones (pDR2.7, pDR7.8, pDR1.6, pDR2), whose positions extend over approximately 100 kb of amplified DNA cloned from LZ (7) (Fig. 2, bottom).

Results of these experiments demonstrated that all four transferents analyzed have acquired multiple copies of the hamster multidrug resistance sequences. Probes pDR2.7 (Fig. 2A and a) and pDR7.8 (Fig. 2B and b) are most species specific and, under the stringent wash conditions used, do not hybridize with any endogenous mouse sequences. Hybridization patterns obtained with these probes clearly show the transfer of Chinese hamster DNA sequences from the amplified domain of LZ in all four clones. The intensity of hybridization is consistent with the presence of approximately 10 to 15 copies of the sequences homologous to these probes. Hybridization patterns obtained with probes pDR1.6 (Fig. 2C and c) and pDR2 (Fig. 2D and d) show the transfer of multiple copies of Chinese hamster multidrug resistance DNA sequences to LTA cells, as well as the presence of homologous mouse multidrug resistance DNA sequences. The level of hybridization of endogenous mouse sequences in the transferents was approximately equal to the level of hybridization observed in LTA controls, indicating that amplification of endogenous mouse multidrug resistance sequences does not contribute to the drug-resistant phenotype of the transferents. To further address these issues, a mouse cDNA complementary to the 5-kb mRNA encoded by the multidrug resistance genomic region was hybridized to the transferent DNA. This cDNA clone, pcDRK, was obtained by screening a mouse cDNA library with genomic probes pDR1.6 and pDR7.8 isolated from the cloned LZ domain (6; D. E. Housman, J. M. Croop, T. Mukayama, I. Roninson, and P. Gros, *in* 8th Bristol-Myers Symposium on Cancer Research, in press). Despite the higher degree of homology of this probe to mouse multidrug resistance sequences, more intense hybridization was observed in the transferents to restriction fragments identical in mobility to fragments of hamster origin (Fig. 2E). Hybridization to fragments of mobility similar to endogenous mouse multidrug resistance sequences was of approximately the same intensity in the transferents and in LTA controls. This result further suggests that amplification of endogenous

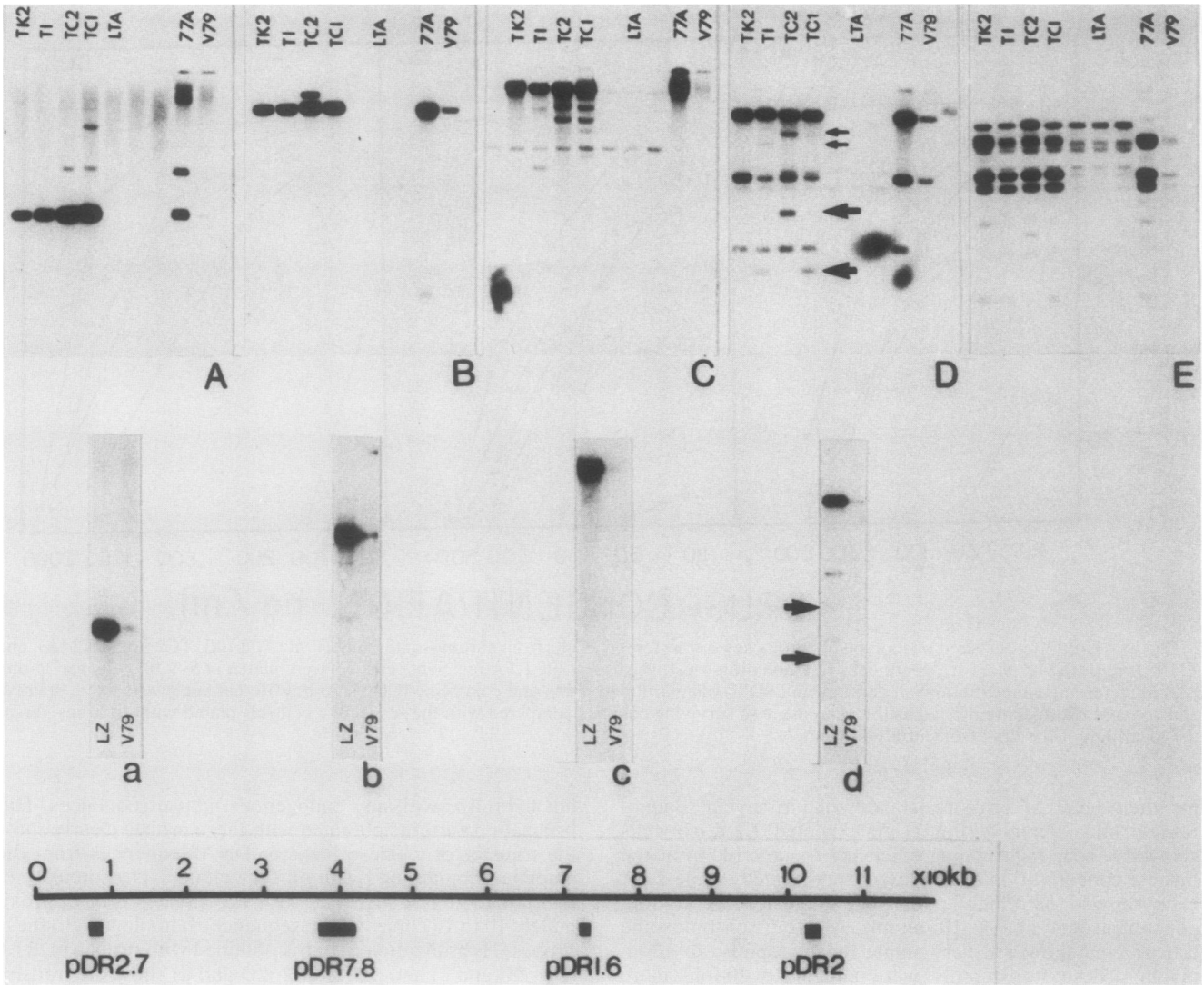

FIG. 2. Southern analysis of genomic DNA from chromosome transferents. The positions of repeat-free subclones pDR2.7 (2.7-kb *Bam*HI fragment), pDR7.8 (7.8-kb *Eco*RI fragment), pDR1.6 (1.6-kb *Bgl*II fragment), and pDR2 (2-kb *Xba*I fragment) isolated from the genomic domain cloned from LZ are presented in the lower section. These subclones were used as hybridization probes to screen Southern blots of DNA from V79 hamster cells and the V79 multidrug-resistant derivative LZ (middle section). DNA (10 μg) was digested with *Bam*HI (a and c) or *Eco*RI (b and d) and probed with pDR2.7 (a), pDR7.8 (b), pDR1.6 (c), or pDR2 (d). Blots were exposed to X-ray films for 16 h. DNA (10 μg) from chromosome transferents TK2, TI, TC2, and TC1 along with three subclones of LTA cells, V79 hamster cells, and the V79 drug-resistant derivative 77A was digested with *Eco*RI (B, D, and E) or *Bam*HI (A and C). Blots were probed with genomic probes pDR2.7 (A), pDR7.8 (B), pDR1.6 (C), pDR2 (D), and a cloned cDNA probe, pcDRK (E). The presence of new hybridizing fragments in the chromosome transferents is indicated by small and large arrows in panels D and d (see text in Results). Blots were exposed to X-ray films for 36 h.

mouse multidrug resistance sequences does not contribute to the drug-resistant phenotype of the transferents.

The levels of drug resistance and the copy number of hamster multidrug resistance genes were significantly lower in the transferents than in the parent cell line LZ. To provide a more appropriate comparison for the transferents, we chose to utilize the hamster cell line 77A as a reference point. This cell line was originally derived by single-step selection in adriamycin at 0.05 μg/ml and has a level of drug resistance similar to that of the transferents (9). 77A has previously been shown to have approximately 10 copies of the hamster multidrug resistance gene (20). When the intensity of the hybridization signal of 77A DNA is used as a reference, it appears that the four clones analyzed received 10 to 15 copies of the hamster gene. Cell line 77A exhibits a drug-resistant phenotype similar to that of the four transferents. The correlation between copy number of the multidrug resistance amplification unit and the drug-resistant phenotype of the four transferents and 77A suggests that overexpression of the multidrug resistance gene is the basis of the drug-resistant phenotype of the transferents.

Genomic DNA analysis of the four chromosome transferents also revealed the presence of an additional set of hybridizing bands (Fig. 2, arrows) which are absent in the phenotypically similar hamster 77A cell line and its parent, V79, and which do not correspond to any LTA-hybridizing bands. These are more readily detectable in the genomic DNA of clones TC1 and TC2. Some of these additional

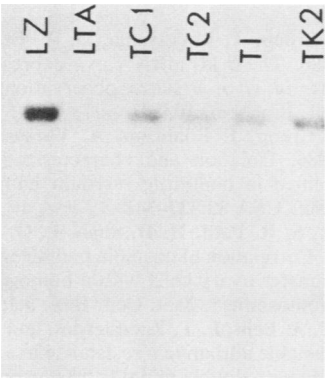

FIG. 3. Northern analysis of cellular RNA from chromosome transferents. Total cellular RNA (10 μg) from mouse LTA cells and chromosome transferents TC1, TC2, TI, and TK2 as well as multidrug-resistant hamster LZ cells (5 μg) was electrophoresed in a denaturing formaldehyde-agarose gel, transferred to a hybridization membrane, and probed with a segment of the mouse cDNA clone pcDRK. The blot was exposed to X-Ray film for 10 h.

bands (large arrows) are present in the donor cell, LZ (this is most evident with probe pDR2). These fragments probably result from complex rearrangements during the process of gene amplification in the selection of LZ cells and are most likely transferred by chromosome-mediated gene transfer. On the other hand, new fragments absent in V79, 77A, and LZ but specific to some of the transferents were detected by all hybridization probes (indicated by small arrows in Fig. 2D). These new bands probably represent rearranged fragments generated either during the chromosome transfer or by subsequent amplification of the transferred sequences. The contribution, if any, of these rearranged copies of the multidrug resistance region to the drug-resistant phenotype cannot be directly determined.

Expression of mRNA in chromosome transferents. To determine whether the transfer of 10 to 15 copies of the hamster gene was associated with overexpression of the 5-kb mRNA, we performed a Northern blotting analysis of total cellular RNA isolated from TC1, TC2, TK2, TI, LZ, and mouse LTA control cells (Fig. 3). To facilitate comparison, 5 μg of LZ RNA, as opposed to 10 μg for all the other cell lines, was electrophoresed in a formaldehyde-containing agarose gel. After transfer to a hybridization membrane, the blot was probed with a subclone of the mouse cDNA clone pcDRK. Hybridization of this probe to an RNA species of approximately 5 kb was observed in all transferents. The mobility of this mRNA species was identical to the mobility of the analogous mRNA species observed in LZ. The intensity of hybridization was many times greater than hybridization to the homologous RNA species of this size present in LTA cells. Hybridization to transferent RNA was significantly less intense than hybridization to LZ RNA. The observed correlation between the transfer of multiple copies of the hamster multidrug resistance gene and the increased expression of the 5-kb mRNA suggests that increased levels of the 5-kb mRNA are due to transcripts of the amplified hamster multidrug resistance DNA sequences.

DISCUSSION

In this report we show that the multidrug-resistant phenotype of LZ hamster cells can be transferred to drug-susceptible mouse LTA cells by chromosome-mediated gene transfer. The multidrug-resistant phenotype in four independent transferents was shown by analysis of genomic DNA to result from the transfer of multiple copies of the hamster gene from the LZ cells rather than from amplification of the endogenous copy of the mouse gene. A comparative analysis shows that, in all four clones tested, the transfer of 10 to 15 copies of the hamster gene was sufficient to produce a multidrug-resistant phenotype. Phenotypic analysis of individual clones selected in adriamycin showed the resistance to be pleiotropic. All four clones tested also demonstrated similar levels of cross-resistance to daunorubicin and colchicine. These results suggest that the DNA sequences transferred are able to confer the complete multidrug-resistant phenotype on recipient cells.

The data presented here are the outcome of a direct comparison between chromosome-mediated gene transfer and DNA-mediated gene transfer in our laboratory. Our results have been consistent with those of other investigators with regard to the low frequency of apparent transfectants obtained via DNA-mediated gene transfer (3) and with regard to the high numbers of endogenous amplificants among multidrug-resistant clones produced by this technique (P. Gros and C. Stanners, unpublished data; D. Shen and M. M. Gottesman, unpublished data). We found that chromosome-mediated gene transfer was more successful than DNA-mediated gene transfer in the transfer of multidrug resistance from LZ cells to LTA cells.

There are several possible reasons for the improved success rate for transfer of multidrug-resistant sequences by chromosome-mediated gene transfer. First, the presence of multiple copies of multidrug-resistant sequences in transfectants appears necessary for the expression of the multidrug-resistant phenotype (16, 18). In DNA transfer, DNA is sheared to segments of 100 kb or less. By contrast, chromosome-mediated gene transfer allows the transfer of contiguous segments of DNA up to 10^7 base pairs in length (approximately 1% of the genome). Amplified multidrug resistance sequences are likely to be arranged in tandem arrays in donor cells such as LZ, and the transfer of long segments of DNA would favor the transfer of multiple copies of multidrug resistance sequences. An additional problem in DNA-mediated gene transfer of multidrug resistance may be the length of the transcription unit within the multidrug resistance region. Previous studies indicate that the transcription unit encoding the 5-kb mRNA in the amplified DNA domain of LZ spans at least 75 kb of genomic DNA. The transfer of a DNA segment of this length by DNA-mediated gene transfer can be inefficient owing to shearing of DNA during DNA preparation or degradation during calcium phosphate precipitation and DNA uptake by cells or both. On the basis of our interpretations, we propose that chromosome-mediated gene transfer should be considered as an alternative to DNA-mediated gene transfer in situations in which gene amplification or large gene size or both are suspected.

The demonstration that transfer of multiple copies of the amplified DNA domain of LZ cells can confer multidrug resistance on LTA cells raises the issue of the identification of the protein species encoded by the amplified domain. Analysis of the cellular RNA indicates that multidrug resistance acquired by chromosome-mediated gene transfer is paralleled by increased expression of the 5-kb mRNA. These results confirm our previous findings on the central role of this mRNA in the multidrug-resistant phenotype of various hamster, mouse, and human cell lines (6, 7, 20a). Ling and co-workers (10, 17) have isolated a cDNA clone which

encodes a segment of gp170, a membrane glycoprotein whose overexpression has been correlated with multidrug resistance in various cell lines. This cDNA probe detected both gene amplification and overexpression of a 4.5-kb mRNA in multidrug-resistant hamster cells (10). The relationship of the 5-kb mRNA in studies from our (7) and other (10; Roninson et al., in press) laboratories to gp170 is not yet clear. Both the size of the specific mRNA (5 kb) and the fact that LZ cells used in our study express high levels of gp170 (P. Gros, D. E. Housman, and V. Ling, unpublished data) argue in favor of a direct relationship between the cloned gene and gp170.

The study presented here represents a further step in a general multistep approach designed to identify, isolate, and characterize amplified sequences in mammalian cells (7; Housman et al., in press). In-gel renaturation techniques (19) can be used as a first tool to identify genes amplified by 30 to 50 copies and to present a minimum catalog of the restriction fragments overlapping the amplification unit. A modification of the original technique (20) can then be used to directly clone one of the restriction fragments from within the amplified unit. Chromosome-walking techniques in addition to in-gel renaturation analysis may then be used to rapidly isolate and characterize from cosmid genomic libraries large segments (≥150 kb) of the amplification unit (7). Northern analysis can then be used to identify transcription products from the cloned domain (6, 7). The biological activity of the amplified sequences can then be tested in transfection assays by chromosome-mediated gene transfer.

The results presented here provide further support for the view that multidrug resistance is due to overexpression of the gene located within the boundaries of the amplified DNA domain of LZ cells. The overexpression of the 5-kb mRNA transcript encoded by the multidrug resistance region is observed in all four transferents. While these results are consistent with a primary role for these sequences in conferring drug resistance, definite proof will come from drug resistance transfer experiments with cloned genomic or cDNA sequences.

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