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Overexpression and Amplification of Five Genes in a Multidrug-Resistant Chinese Hamster Ovary Cell Line

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Multidrug-resistant cells are cross-resistant to a wide range of unrelated drugs, many of which are used in cancer chemotherapy. We constructed a cDNA library from RNA of the multidrug-resistant Chinese hamster ovary cell line CH^RC5. By differential screening we isolated cDNAs derived from mRNAs that are overexpressed in this cell line. The cDNAs could be grouped in five classes on the basis of transcript lengths detected in RNA blots. We infer that each class codes for a separate protein. The corresponding genes are amplified 10 or 30 times in CH^RC5 DNA, providing an explanation for the constitutive overexpression found in this cell line. Despite differential amplification, the genes may be linked in one large amplicon as indicated by the hybridization analysis of large fragments of CH^RC5 DNA separated by pulsed field gradient gel electrophoresis. Therefore, some of these genes might be fortuitously coamplified and not contribute functionally to the resistant phenotype. It is also possible, however, that genes involved in drug resistance are clustered. One of our clones cross-hybridized with the recently described cDNA pCHP1 (J. R. Riordan, K. Deuchars, N. Kartner, N. Alon, J. Trent, and V. Ling, Nature [London] 316:817–819, 1985) encoding part of the 170-kilodalton P-glycoprotein, a protein which is frequently overproduced in multidrug-resistant cells. The nature of the four other genes is still unknown. Sequences of four of the five classes of cDNAs are conserved in mouse and human DNA.

Acquisition of multidrug resistance by malignant cells may limit the effectiveness of cancer chemotherapy, because treatment with a cytostatic drug can result in cross-resistance to a wide range of other drugs (4, 9, 21, 30). The drugs involved in multidrug resistance in model cell lines, such as colchicine, adriamycin, and actinomycin D, differ in mode of action, target, and chemical structure. The observed pattern of cross-resistance reflects the selective drug and the cell type employed (5, 20, 30). In general, the highest increase in resistance is found for the drug used in selection, although there are exceptions. The apparent complexity of the multidrug-resistant phenotype is suggestive of a multicomponent system. Resistance is the result of limiting the intracellular drug concentration by reduced import (8), enhanced efflux (17), or perhaps both (see reference 30).

The 170-kilodalton (kDa) P-glycoprotein, overproduced in many multidrug-resistant cell lines (18, 19), is a potential component of the mechanism causing resistance. The degree of overproduction correlates well with that of resistance as was shown both in a series of cell lines obtained by stepwise selection for increased resistance (18, 31) and in revertants with reduced drug resistance (5, 21). Smaller proteins have also been associated with multidrug resistance. A 19-kDa cytosolic protein (V19) is overproduced in a Chinese hamster lung cell line selected for resistance with vincristine (25). Analogous small proteins are overproduced in resistant mouse and human cell lines (25, 26). Their overproduction depends on the drug used for selection, however. In addition, the putative mouse homolog (21 kDa) of V19 has recently been reported to disappear after prolonged culture, even though multidrug resistance was retained (24). The loss of proteins (72 and 75 kDa) has also been found in a multidrug-resistant cell line (27), completing the spectrum of altered protein levels which have been associated with multidrug resistance. How each of these proteins contributes to the ability of the cell to maintain a low intracellular drug concentration is unknown.

The overproduction of proteins, and in particular those involved in drug resistance in mammalian cells, is often the result of gene amplification (see references 7, 14, 21, 33, 36). Homogeneously staining regions or double minute chromosomes, both known to carry amplified DNA, have been observed in a variety of multidrug-resistant cell lines (3, 5, 12, 29). The degree of amplification, reflected by the size of the homogeneously staining region or the number of double minute chromosomes, can be correlated with the level of P-glycoprotein overproduction (5, 29, 31). Recently, a cDNA clone encoding part of the P-glycoprotein has been isolated from an expression library (29). This clone hybridizes to a homogeneously staining region, and, in line with the cytological observations, the degree of amplification corresponds to the level of resistance. Although overproduction of the 170-kDa P-glycoprotein remains the most consistent feature, homogeneously staining regions have also been observed in multidrug-resistant cell lines without an apparent overproduction of the P-glycoprotein (24).

The disparity in altered protein levels and gene amplification suggests that several proteins contribute to the multidrug-resistant phenotype. To identify genes encoding proteins involved in multidrug resistance, we constructed a cDNA library from the resistant chinese hamster ovary (CHO) cell line CH^RC5 and isolated cDNA clones derived from overexpressed transcripts by differential hybridization. In this paper we describe the initial characterization of cDNAs corresponding to at least five genes that are overexpressed and amplified in the resistant cell line.

MATERIALS AND METHODS

Cell culture. The CHO cell lines AUXB1 and CH^RC5 were cultured as described previously (22) without colchicine.

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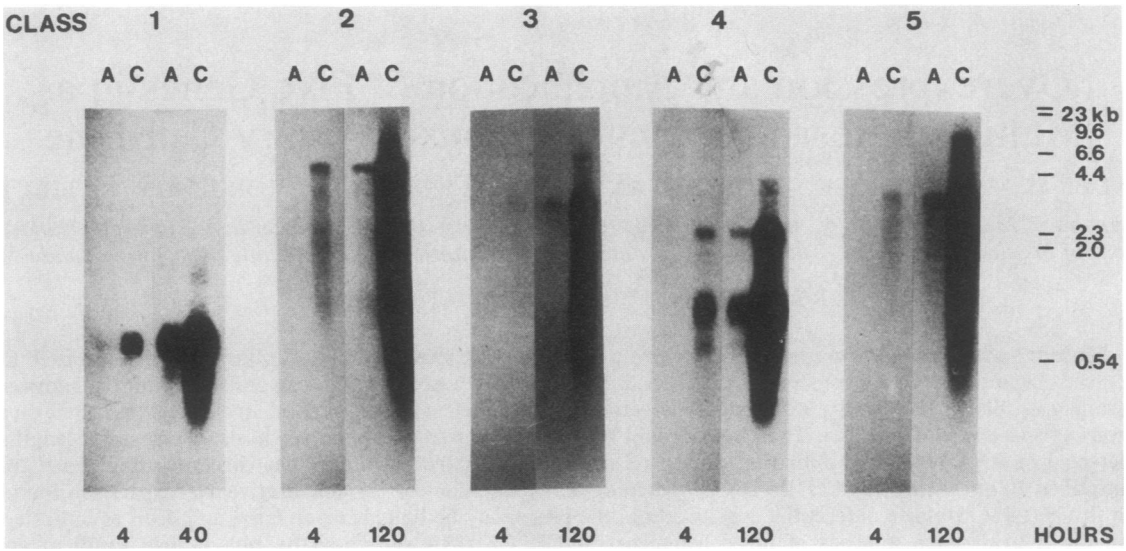

FIG. 1. Overexpression of five different groups of mRNAs identified by the differential screening of a cDNA library made from the CH^{R}C5 cell line. Size-fractionated poly(A)$^+$ RNA from the parental cell line AUXB1 (A) and from the resistant cell line CH^{R}C5 (C) was hybridized to representative cDNAs. Autoradiograms of the filters are shown with two exposures chosen such that the signal from the AUXB1 RNA on the long exposures (40 or 120 h) is comparable to that from the CH^{R}C5 RNA on the short exposures (4 h). The samples were alternated with the size marker, denatured *Hin*dIII-digested phage lambda DNA, which was detected with nick-translated phage lambda DNA. The cDNA used for each class is indicated as prototype cDNA in Table 1.

Persistence of the multidrug-resistant phenotype was tested by culturing a sample of the harvested cells with 10 μg of colchicine (Sigma Chemical Co., St. Louis, Mo.) per ml. For the induction experiments, colchicine was added to the culture medium 48 h before cell harvest and RNA isolation, at the sublethal concentrations of 20 ng/ml for the AUXB1 cells and 5 μg/ml for the CH^{R}C5 cells. The mouse cell line L1210 and the human cell line COLO 320 DM (1) were grown in RPMI 1640 medium. The media were supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 U of penicillin per ml, and 50 μg of streptomycin per ml. Cell harvest and transfer was by standard trypsinization procedures.

RNA isolation, fractionation, and blotting. RNA was isolated from cultured cells by the LiCl-urea method (2), DNA was removed by DNase treatment, and poly(A)$^+$ RNA was separated on an oligo(dT)-cellulose column (see reference 23). RNA was size fractionated by electrophoresis in 1.4% formaldehyde-agarose gels, run for 20 h at 2 V/cm, and treated with alkali, followed by neutralization and a wash in 20× SSC (3 M NaCl, 0.3 M sodium citrate, pH 6.4) before blotting to nitrocellulose (0.45-μm pore size; Schleicher & Schuell, Inc., Keene, N.H.) (see reference 23). Hybridization conditions were as described below for Southern blots.

Construction of cDNA library and differential screening. The synthesis of double-stranded cDNA was essentially as described by Gubler and Hoffman (13), using reverse transcriptase from Anglian Biotechnology, Ltd. The double-stranded cDNA was dC tailed and annealed to the *Pst*I-cleaved and dG-tailed pUC9 vector (Pharmacia P-L Biochemicals). Transformants of *Escherichia coli* DH1 (15) were plated on nitrocellulose, and four replica filters were prepared for colony hybridization (16). The library contained approximately 60,000 colonies with inserts mostly ranging from 500 to 1,500 base pairs. The four sets of replica filters were hybridized to single-stranded cDNA probes of high specific activity synthesized from both AUXB1 and CH^{R}C5 poly(A)$^+$ RNA by using random primers (see reference 23) followed by alkaline degradation of the RNA (20 min in 0.2 N NaOH at 70°C). The filters were hybridized for 100 h under the conditions described below for Southern blots, with the addition of 10 mM sodium PP$_i$. After autoradiography the signal was removed (30 min in 0.1 N NaOH), and the filters were rehybridized with the other cDNA probe. Colonies showing a stronger signal on all four replica filters when hybridized with the CH^{R}C5 cDNA probe were chosen for further analysis.

DNA preparation, blotting, and nick translation. Genomic DNA from cultured cells was prepared as described previously (23), and plasmid DNA was prepared from *E. coli* by alkaline lysis (6). Genomic DNAs were digested with restriction endonucleases (see reference 23). Digests were considered complete if bacteriophage lambda DNA added to a sample of the incubation mixture was completely digested. The DNA fragments were size fractionated in 0.5% agarose gels, run for 40 h at 1.5 V/cm, acid degraded (20 min in 0.25 N HCl), and blotted to nitrocellulose (35). The filters were prehybridized for 60 min at 65°C in 3× SSC–0.1% sodium dodecyl sulfate–5× Denhardt solution–50 μg of denatured salmon sperm DNA per ml (23). The hybridizations were conducted for 16 h at 65°C with gel-purified and nick-translated (28) cDNA fragments. Unless otherwise specified, the final wash was for 30 min at 65°C in 0.1× SSC. Autoradiography was at −70°C on Kodak XAR film with Du Pont Cronex Lightning-Plus screens.

Pulsed field gradient gel electrophoresis. Pulsed field gradient gel electrophoresis was done essentially as described by Schwartz and Cantor (34). The gels were run for 20 h at 5°C in an electrophoresis chamber (20 by 20 cm) with an inhomogeneous field of 350 V and a perpendicular homogeneous field of 140 V alternating every 35 s. The mammalian cells were harvested by trypsinization, washed, suspended in phosphate-buffered saline adjusted to pH 7.2, mixed 1:1 with 1% low-melting-temperature agarose (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) in phosphate-buffered saline to a final cell concentration of 2×10^7 cells per ml, and solidified in molds so as to fit the gel slots. The

TABLE 1. Summary of data obtained with cDNAs derived from mRNAs overexpressed in the multidrug-resistant CH^RC5 cell line[a]

Class	No. of clones	cDNA prototype	Total in clone (bp)	Transcripts (nt)	DNA amplification (fold)	End products (kb) with:	
						*Sac*II	*Sfi*I
1	1	cp19	540	750	10	440	125
2	4	cp28*	3,500	4,500	10 and 30	125, 440	120, 160, 280
3	1	cp16	680	3,400	30	275, 440	80
4	11	cp6	2,400	1,000, 2,500	30	>650	190, 300
5	1	cp30	2,600	3,600	30	>650	190, 300

[a] The cDNAs were grouped in classes, and the total cloned segment is given in base pairs next to the size of the major transcripts detected on RNA blots. The cDNA cp28, which cross-hybridizes with the 170-kDa P-glycoprotein-encoding cDNA pCHP1, is indicated by an asterisk.

agarose blocks were treated with 1 mg of proteinase K (E. Merck AG, Darmstadt, Federal Republic of Germany) per ml–0.5 M EDTA (pH 9.5)–1% sodium *N*-lauroyl sarcosinate for 48 h at 50°C and prepared for restriction enzyme digestion by washing in 50 ml of 10 mM Tris chloride (pH 7.5)–10 mM EDTA–0.1 mM phenylmethylsulfonyl fluoride (Merck), followed by a 60-min wash in 15 ml of the restriction enzyme buffer and 0.1 mM phenylmethylsulfonyl fluoride. The *Sac*II (New England BioLabs, Inc., Beverly, Mass.) and *Sfi*I (New England BioLabs) digests were performed at 37 and 50°C, respectively, with approximately 10 μg of DNA embedded in 50-μl agarose blocks added to 50 μl of buffer with the enzyme concentrations and for the times specified in the figure legends. The digests were stopped by washing the blocks in 10 mM Tris chloride (pH 7.5)–10 mM EDTA–0.1 mM phenylmethylsulfonyl fluoride. After electrophoresis the gel slots were sealed with 1% agarose, and the DNA was stained with 2.5 μg of ethidium bromide per ml. The blotting and hybridization conditions were as described in the previous section. Intact chromosomal DNA from *Trypanosoma brucei* M249 was prepared for pulsed field gradient gel electrophoresis as described by Van der Ploeg et al. (37). Multimers of phage lambda were prepared by solidifying 10 μg of DNA per ml in 0.5% low-melting-temperature agarose blocks and allowing the cohesive ends to anneal for 14 days at room temperature in 10 mM Tris chloride (pH 8.0)–10 mM EDTA.

RESULTS

Isolation and characterization of cDNA clones complementary to mRNAs overexpressed in a multidrug-resistant cell

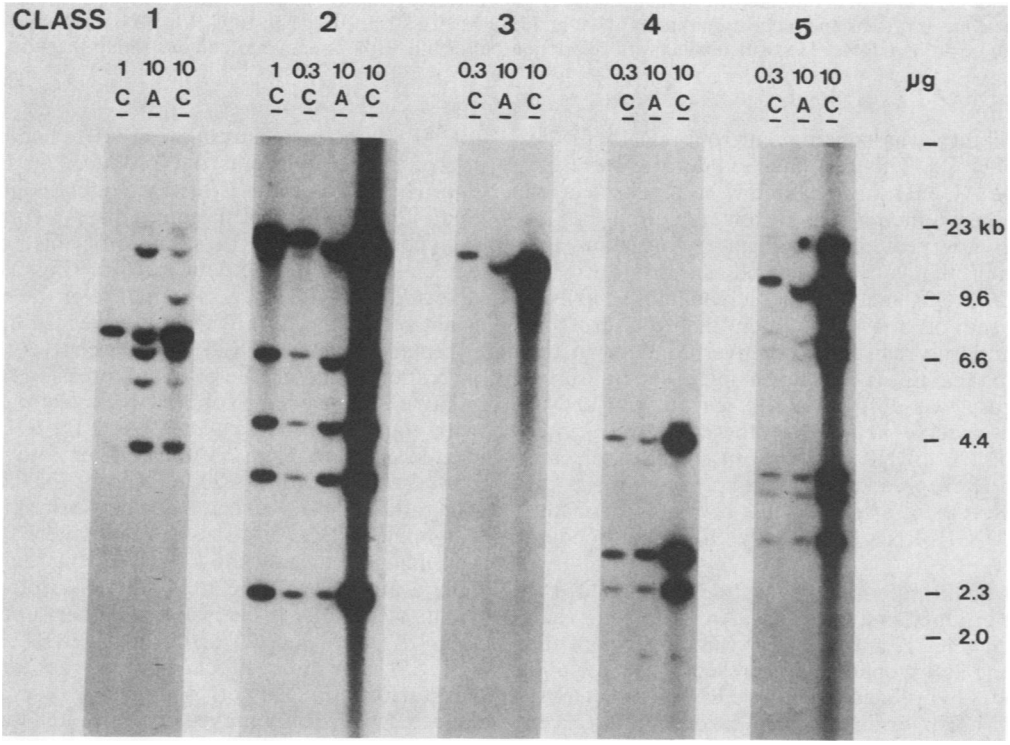

FIG. 2. Amplification of genes corresponding to overexpressed mRNAs. *Eco*RI-digested genomic DNAs from the parental cell line AUXB1 (A) and from the resistant cell line CH^RC5 (C) were hybridized to representative cDNAs (Table 1). The amount of DNA is indicated above each lane. Dilutions of the CH^RC5 DNA were chosen such that the signal from the amplified *Eco*RI fragments equals the parental level in AUXB1 DNA. Differentially amplified *Eco*RI fragments are detected with the probe encoding part of the mRNA for the 170-kDa P-glycoprotein (class 2; cp28). This is illustrated by the two dilutions of CH^RC5 DNA (0.3 and 1 μg) needed to equal the signal from AUXB1 DNA (10 μg). The size marker was *Hin*dIII-digested phage lambda DNA.

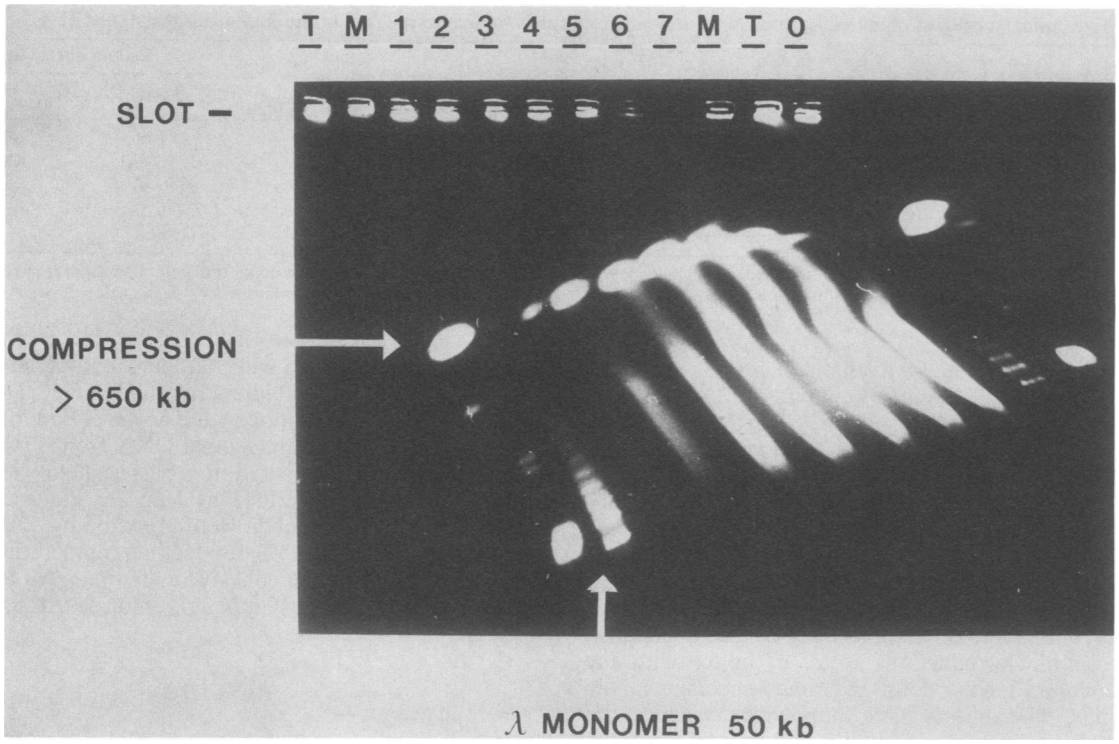

FIG. 3. Restriction fragments of hamster DNA size fractionated by pulsed field gradient gel electrophoresis. The gel was run for 20 h with a pulse duration of 35 s and stained with ethidium bromide. Fluorescence in the slot is from DNA fragments that are presumably immobile owing to their large size. The compression zone represents DNA fragments larger than 650 kb. Smaller fragments migrate with an approximately inverse relation to their size as shown by the marker lanes containing multimers of phage lambda DNA (M). For comparison, the chromosomes of *T. brucei* M249 are shown in flanking lanes (T). The center lanes (1 to 7) contain *Sfi*I digests of CH^RC5 DNA with increasing enzyme concentrations and incubation times (3 U, 1 h; 6 U, 1 h; 10 U, 1 h; 10 U, 2 h; 10 U, 4 h; 50 U, 16 h; and 100 U, 16 h). The right-hand lane (0) contains CH^RC5 DNA incubated with restriction buffer but without enzyme, demonstrating that the DNA is virtually undegraded.

line. A cDNA library was constructed from mRNA of the CHO cell line CH^RC5. This cell line was derived from the parental cell line AUXB1 through stepwise clonal selection for colchicine resistance (22). It has acquired cross-resistance to a variety of other cytostatic drugs, and the 170-kDa P-glycoprotein is overproduced as a result of gene amplification (29). Our method for screening the cDNA library was based on the assumption that the proteins relevant for multidrug resistance are overproduced in the resistant cells and that this is reflected in increased transcript levels. The library was differentially screened with cDNA probes of high specific activity synthesized from either CH^RC5 or AUXB1 mRNA. Of the 40 initially selected recombinants, 18 represented overexpressed mRNAs. These clones were then tested on blots of size-fractionated CH^RC5 and AUXB1 RNA to identify the corresponding transcripts.

On the basis of the hybridization patterns obtained, the clones could be grouped in five classes. An example of each class is shown in Fig. 1, and the approximate sizes of the major hybridizing RNA species are presented in Table 1. Most of the probes hybridized to multiple RNAs, but cDNAs of one class did not hybridize to cDNAs of another class, with the possible exception of a 3,400-nucleotide (nt) RNA, which was the main transcript detected by the class 3 probe and which also weakly hybridized to the class 2 probes. We infer from these results that the five classes of cDNAs correspond to five different genes.

Riordan et al. (29) have recently isolated a cDNA clone (pCHP1) containing part of the mRNA encoding the 170-kDa P-glycoprotein which is overproduced in the CH^RC5 cell line. This cDNA cross-hybridized only to our class 2 cDNAs (data not shown), and therefore class 2 corresponds to the P-glycoprotein gene. In support of this, class 2 cDNAs detected a single mRNA of 4,500 nt (Fig. 1) similar to that detected by pCHP1 (29). The nature of the other four genes is not yet known. The five genes were also transcribed in the parental cell line AUXB1, but at relatively low levels. An indication of the degree of overexpression follows from the relative time needed to obtain an exposure of CH^RC5 RNA with equal intensity to that of AUXB1 RNA (Fig. 1). The degree of overexpression ranged from 5 to 10 times for the class 1 cDNA (cp19) to 20 to 30 times for the other cDNAs. The ratio of the 1,000- and 2,500-nt transcripts recognized by cp6-related cDNAs (class 4) was unaltered in the resistant cell line. We do not know whether the longer transcript is only a precursor of the 1,000-nt transcript. Control experiments were done to determine whether subtoxic concentrations of colchicine (20 ng/ml for AUXB1 and 5 μg/ml for CH^RC5) had a direct effect on the expression levels. Induced expression of genes corresponding to our cDNAs was not detected with RNA harvested 48 h after the application of colchicine (data not shown). The level of expression thus appears to be fixed in these cell lines, and overexpression is constitutive upon acquiring resistance.

Transcription of overproduced mRNAs from amplified genes. To verify whether increased mRNA levels are due to an increased copy number of the corresponding genes, we

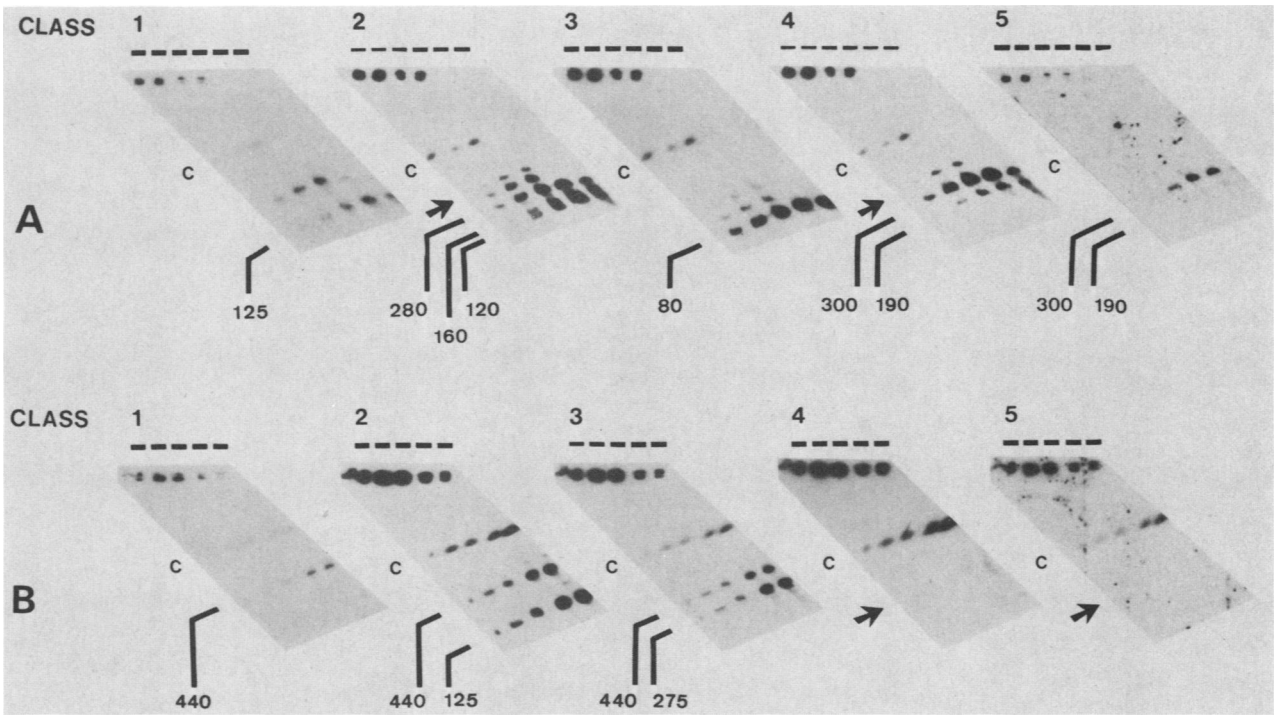

FIG. 4. Series of partial *Sfi*I (A) and *Sac*II (B) digests of CH^{R}C5 DNA hybridized with representative cDNAs. The cDNAs used are indicated as prototype cDNA in Table 1, except for cp20 (class 2) which was used as a probe instead of cp28. The digested DNA was separated by pulsed field gradient gel electrophoresis, as shown in Fig. 3 for the *Sfi*I digests, and transferred to nitrocellulose. The positions of the slots (bars) and the compression zone (C) are indicated. The sizes of the complete products of digestion, given in kilobases, were estimated by using the flanking lanes of lambda DNA multimers made visible with the ethidium bromide stain (Fig. 3). In panel A the arrows indicate the 400-kb *Sfi*I partial digestion products detected both by class 2 and 4 cDNAs, while in panel B they indicate that neither of the class 4 and 5 cDNAs detects a 440-kb *Sac*II product. Partial digestion was obtained with a series of increasing enzyme concentrations and incubation times. For the *Sfi*I digests (A) these were 6 U, 1 h; 10 U, 1 h; 10 U, 2 h; 10 U, 4 h; 50 U, 16 h; and 100 U, 16 h; and for the *Sac*II digests (B) these were 2.5 U, 1 h; 5 U, 1 h; 10 U, 1 h; 10 U, 2 h; and 10 U, 4 h. The blots were rehybridized sequentially with each of the cDNA probes after removal of the signal from the previous hybridization by alkaline treatment.

hybridized the cDNAs isolated by a differential screening method to *Eco*RI-digested genomic DNA from the resistant CH^{R}C5 and the parental AUXB1 cell lines. Each probe detected fragments amplified in the resistant cell line (Fig. 2). The level of amplification was determined by comparing dilution series of *Eco*RI-digested CH^{R}C5 DNA with undiluted digested AUXB1 DNA. The exact DNA concentrations on the blot were rechecked with a probe for the single-copy dihydrofolate reductase gene (data not shown). Two levels of amplification were found: 10 times for class 1 and 30 times for the other four classes of cDNAs. Some of these cDNAs hybridized to a remarkably large number of *Eco*RI fragments, for instance, a 1,700-base-pair cDNA fragment from cp30 (class 5) recognized up to nine amplified *Eco*RI fragments with a total length of 44 kilobases (kb). In addition, some of the cDNAs hybridized with nonamplified fragments. The cDNA cp19 (class 1) is the clearest example, hybridizing to at least four nonamplified and two amplified fragments. The class 2 clones, which correspond to the P-glycoprotein, hybridized to many fragments and detected differential amplification in CH^{R}C5 DNA: 10 times for the 3.5-, 4.5-, and 6.3-kb fragments and 30 times for the 2.2- and 17-kb fragments (Fig. 2). This indicates that class 2 cDNAs hybridize to at least two genes (29).

Linkage of amplified genes. Gene amplification is often accompanied by coamplification of very large stretches of flanking DNA (10, 36, 38). To investigate whether some or all genes reside on the same amplified unit, we digested the DNA from resistant cells with restriction enzymes that cut infrequently in mammalian DNA and size fractionated the resulting fragments by pulsed field gradient gel electrophoresis (34), which allows separation of very large (100 to 1,000 kb) DNA fragments. Figure 3 shows such an analysis with CH^{R}C5 DNA digested with *Sfi*I. Some of the DNA fragments were presumably too large and were trapped in the slot. Other large DNA molecules comigrated in a band referred to as the compression zone. The mobility of the smaller fragments was approximately linearly related to their size as demonstrated by the ladder of lambda multimers used as size markers. Because of the gradient field, the starting position in the gel influenced the degree of separation of the larger molecules.

The infrequently cutting restriction enzymes *Sfi*I and *Sac*II were used in a series of increasing enzyme concentrations and incubation times to distinguish complete from partially digested fragments. The same blots were rehybridized with probes for each of the five genes (Fig. 4). The class 4 and 5 cDNAs (cp6 and cp30) yielded identical patterns with *Sfi*I and with *Sac*II digests. Both probes hybridized with 190- and 300-kb *Sfi*I fragments, strongly suggesting that the corresponding genes are linked and coamplified. Hybridization with the cDNAs derived from the other amplified genes yielded unique, but complicated, patterns of *Sfi*I partials, preventing an unambiguous assignment to common amplified units. In contrast, the patterns with the *Sac*II digests were relatively simple and allowed a more conclusive link-

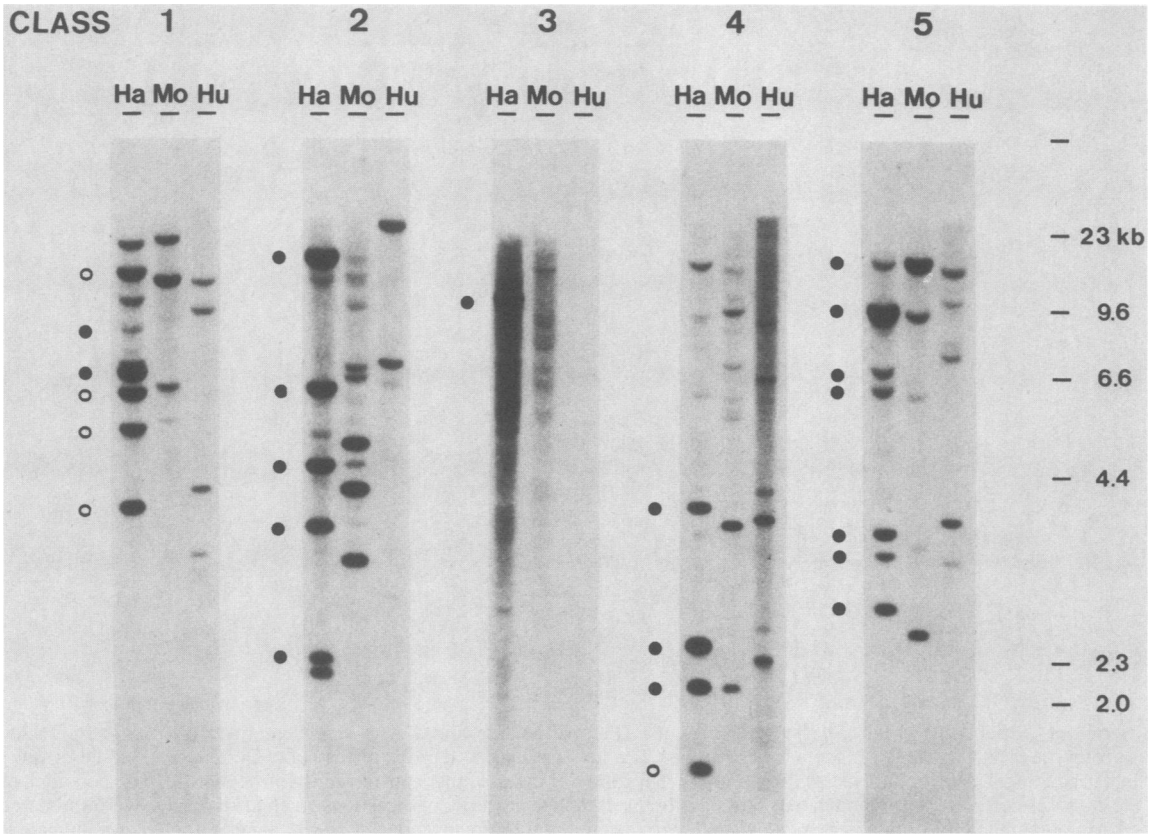

FIG. 5. Sequence conservation of amplified hamster genes in mouse and human DNA. *Eco*RI-digested DNAs (10 μg per lane) from the drug-sensitive hamster AUXB1 (Ha), mouse L1210 (Mo), and human COLO 320 DM (Hu) cell lines were hybridized to cDNA probes that represent the five genes that are amplified in the CH^RC5 DNA (Table 1) and washed at reduced stringency (1× SSC, 65°C). *Eco*RI fragments that are amplified in the CH^RC5 cell line are indicated by a filled circle next to the lanes containing the parental DNA (AUXB1). Nonamplified fragments that remain visible when the blot is washed at 65°C in 0.1× SSC are indicated by an open circle. The size marker is *Hin*dIII-digested phage lambda DNA.

age analysis. Hybridization of class 1, 2, and 3 cDNAs (cp19, cp20, and cp16, respectively) to a 440-kb band indicated that the corresponding genes reside at least in part on a common segment of amplified DNA. The recognition of an additional 125-kb fragment by class 2 and a 275-kb fragment by class 3 cDNAs could be due, for instance, to rearrangements during amplification giving rise to novel *Sac*II fragments. Hybridization with the class 4 and 5 cDNAs was restricted to the slot and the compression zone in this digest, showing that these genes are located on *Sac*II fragments of >650 kb. Taken together, these results indicate that the amplified genes are present on two segments of DNA: one contains class 1, 2, and 3 genes, and the other contains class 4 and 5 genes. Two indirect arguments suggest that these clusters are part of one large amplicon. First, we detected a 400-kb partial *Sfi*I digestion product that appeared to hybridize to class 2 and 4 cDNA probes (Fig. 4A). This indicates that these genes are adjacent. Second, quantitation of the gene amplification showed that there are two levels of amplification (Table 1), confirming the in-gel renaturation studies of Roninson et al. (32), but these two levels did not parallel the two gene clusters identified. Some of the fragments identified by class 2 cDNA probes and the fragment hybridizing to the class 3 probe were amplified 30 times, like the amplified DNA hybridizing to the cDNA probes of class 4 and 5. This would be rather a coincidence if genes 4 and 5 belonged to a different amplicon from genes 2 and 3. The simplest interpretation of these data is that all five genes are adjacent in hamster DNA, that early amplification events led to a 10-fold amplification of these genes, and that subsequent rounds of amplification resulted in the additional amplification of genes 2 through 5. The cDNA probes of class 2 appeared to hybridize to at least two, most likely adjacent genes, one amplified 10 times and the other amplified 30 times, in agreement with the proposal by Riordan et al. (29) that the P-glycoprotein is encoded by members of a gene family.

The *Sac*II digests in Fig. 4A can be interpreted in only two ways. Either the fragment of >650 kb that is recognized by class 4 and 5 cDNAs and is located in the compression zone is an end product of digestion not recognized by class 1 through 3 cDNAs or it is a partial digestion product that includes the 440-kb fragment recognized by class 1 through 3 cDNAs. In the latter case class 4 and 5 cDNAs should hybridize to a second fragment which is shorter by 440 kb. The DNA blots clearly show that this second fragment, if it exists, must be greater than 650 kb in length as well. Therefore, the result of both interpretations is that the wild-type DNA fragment amplified in the CH^RC5 line must be at least 1,100 kb in length.

Sequence conservation of amplified genes. Sequence conservation of the genes was tested by hybridizing the hamster cDNAs to Southern blots of *Eco*RI-digested genomic DNA from mice (L1210) and humans (COLO 320 DM). Cross-hybridization could be detected for each of the cDNAs

except for class 3 (cp16) to human DNA (Fig. 5). The reduced stringency of the final washes (1× SSC, 65°C) used here also revealed several extra fragments in the wild-type hamster genome (AUXB1) not detected with 0.1× SSC washes (Fig. 2). Especially, the class 3 cDNA cp16 hybridized to a large number of fragments in the AUXB1 digest, in addition to the prominent 17-kb band. These repetitive sequences are conserved in mouse, but not in human, DNA. It is possible that the repetitive sequence is present in the 3'-untranslated region of the mRNA and that cDNA cp16 does not contain coding sequences. In that case the lack of hybridization with this probe to human DNA would still be compatible with the conservation of coding sequences for this gene. The relatively high level of sequence conservation found with the other cDNAs suggests that the proteins they encode are essential components of mammalian cells.

DISCUSSION

At least five genes were both amplified and overexpressed in the multidrug-resistant cell line CH^RC5. Each of these genes was also transcribed at a low level in the sensitive Chinese hamster ovary cells (AUXB1). One of the genes encoded the 170-kDa P-glycoprotein, a membrane protein overproduced in CH^RC5 cells. Several lines of evidence firmly link overproduction of this protein to multidrug resistance. It is overproduced in many of the resistant cell lines analyzed thus far (5, 19, 20); the degree of overproduction correlates with the degree of drug resistance (5, 18, 20); and transfer of CH^RC5 DNA into mouse L cells leads to overproduction of the P-glycoprotein and resistance of the host cell (11, 31). Moreover, the 170-kDa P-glycoprotein is the only one of the five genes consistently amplified in each of three other multidrug-resistant hamster cell lines (DC3F/VCRd-5L, -/ADX, and -/DMXX) recently analyzed (M. De Bruijn, A. Van der Bliek, P. Borst, and J. L. Biedler, unpublished data).

As our pulsed field gradient gel analysis suggests that the five genes studied here are adjacent, only the P-glycoprotein gene(s) may have been selected for by colchicine and the others may only have shared in the amplification, because the initial amplicon was so large. Since functionally related genes are often clustered in animal cells, it is also possible, however, that more than one of these genes can contribute to resistance. Sequence analysis, transfer of each gene separately to suitable host cells, and attempts to inhibit expression of each gene in the CH^RC5 cells by antisense RNA will be used to settle the issue. Even if only one of the five genes contributes to drug resistance, the gene products of coamplified genes may provide suitable markers for detecting gene amplification or targets for attack by antibodies or cytotoxic T cells. With this in mind, we are analyzing which of these five genes is amplified in multidrug-resistant cells of human origin, besides the gene for the P-glycoprotein.

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