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Amplification and Expression of Genes Associated with Multidrug Resistance in Mammalian Cells

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In multidrug resistance, which is observed clinically and in tissue culture, cells that are challenged with certain cytotoxic drugs develop resistance not only to the selective agent but also to other, seemingly unrelated, agents. The multidrug-resistant phenotype is associated with DNA sequence amplification and with the overproduction of a number of cytosolic and membrane glycoproteins. The differential amplification and altered expression of at least two related genes, termed multidrug-resistant associated genes has been shown in multidrug-resistant Chinese hamster cells. In multidrug-resistant mouse and human cells, genes homologous to those in Chinese hamster cells are also amplified. The level of expression of these genes varied and did not correlate with their copy number. Furthermore, in Chinese hamster cells, the development of resistance to a single drug and multidrug resistance were closely related, but uncoupled, events. The overexpression of the multidrug-resistant genes was better correlated with the degree of resistance to the selective agent than it was with the extent of multidrug resistance.

THE ESTABLISHMENT AND MAINTENANCE of drug resistance in mammalian cells is mediated by the overexpression of specific genes (*1*). Generally, the drugs irreversibly bind to specific housekeeping enzymes, and resistance is achieved by the overproduction of these target enzymes due to amplification of the genes that encode them (increased gene dosage). The RNA expression is generally less than or equal to, but not greater than, the degree of amplification, and the resulting phenotypes are monospecific; the cells are resistant only to the selective drug or its analogue.

In contrast, cytotoxic drugs that interact with structural proteins or DNA, rather than with housekeeping enzymes, elicit a more complicated response (*2–4*). Cells become refractory to both the selective agent and other, seemingly unrelated, compounds. This more general cross-resistance is apparently mediated by alterations in membrane transport that decrease the intracellular accumulation of these agents (*5*). The phenotypes of these multidrug-resistant cells are complex. (i) Independently selected cell lines display different levels of cross-resistance to the same drugs. (ii) Multidrug-resistant cells contain increased amounts of membrane glycoproteins such as gp150-180 (*6*), p-glycoprotein (*7*), gp180 (*8*), or P180 (*9*); the overproduction of p-glycoprotein in Chinese hamster cells is mediated by gene amplification (*10*). (iii) Some multidrug-resistant cells overproduce cytosolic proteins such as V19 (*11*) in addition to membrane glycoproteins. (iv) Tumorigenic cells selected for multidrug resistance display a reverse transformed phenotype and cannot cause tumors in animals (*12, 13*). The variability and complexity of the multidrug-resistance phenotype suggest that this clinically relevant form of drug resistance may involve a change in gene expression at more than one genetic locus.

We used vincristine-resistant sublines of the Chinese hamster lung (CHL) cell line DC-3F for our studies (*2*) because these cells contain cytological manifestations of gene amplification, such as homogeneously staining regions (HSR) and abnormally banding regions (ABR) (*13, 14*), and elevated gp150-180 (*13*) and V19 (*11*). We could then analyze these cells with methods optimized for the detection of amplified DNA sequences (*15, 16*). We identified at least two related genes, the amplification and overexpression of one or all of which was associated with the multidrug-resistant phenotype. Genes homologous to these were also amplified and overexpressed in multidrug-resistant mouse and human cell lines. In contrast to other drug-resistant cell systems, the level of expression of these genes could exceed that of their amplification and, although this increased expression may correlate with the increase in resistance to the

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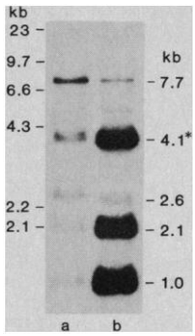

Fig. 1. Southern blot analyses with a cloned cDNA, p5L-18, as probe. The cDNA was prepared from cytoplasmic polyA$^+$ RNA, converted to double-stranded cDNA, and cloned by the poly(dG-dC) tailing method (*21*). Recombinant colonies were transferred to nitrocellulose (*22*), incubated in 6× standard saline citrate (SSC), 4× Denhardt's, denatured salmon sperm DNA (15 μg/ml) for 3 hours at 65°C, then hybridized with [^{32}P]DC-3F C_0t 10 to 300 DNA (1×10^8 cpm/μg) in 4× SSC, 0.5 m*M* EDTA, and denatured salmon sperm DNA (50 μg/ml) at 65°C for 36 hours. Filters were washed at 65°C in 0.1× SSC and 0.1% sodium dodecyl sulfate (SDS) and exposed to Kodak XAR5 film with an intensifying screen for 16 hours. Filters were then stripped of radioactivity in 0.1*M* NaOH and 2× SSC at 25°C for 30 minutes, washed extensively in 2× SSC at 25°C, incubated as above, hybridized with [^{32}P]VCR^r C_0t(10–300) DNA (1×10^8 cpm/μg), washed, and exposed again under the same conditions. Eight colonies hybridized preferentially with the VCR^r DNA probe; of these, one clone, designated p5L-18, was isolated after a second round of screening. Large-scale preparation of this plasmid was by a modification of the procedure of Norgard (*23*), as described (*21*), except cells were grown for 16 hours in L broth supplemented with ampicillin (100 μg/ml), and were harvested without prior chloramphenicol amplification. p5L-18 was nick-translated (*24*) to a specific activity of 2×10^8 cpm/μg and used as a probe for Southern blot analyses (*20*) of restriction endonuclease digested genomic DNA fractionated on 0.8% agarose gels. Hybridizations were carried out in 4× SSC, 8 m*M* EDTA, and denatured salmon sperm DNA (300 μg/ml), at 68°C for 16 hours; washes were in 0.1× SSC, 0.1% SDS at 68°C. Filters were exposed to Kodak X-OMAT XAR5 film with an intensifying screen for 2 to 24 hours at −80°C. (A) Eco RI–digested genomic DNA from DC-3F cells, 20 μg. (B) Eco RI–digested genomic DNA from DC-3F/VCRd-5L cells, 10 μg. Hind III–digested λ DNA was used as a size marker.

selective agent, it did not result in a commensurate crossresistance.

C_0t fractionation of DNA from cells that amplify genomic sequences allows the preparation of DNA samples that are highly enriched in those amplified sequences (*15*). When the C_0t 10 to 300 fraction taken from cells that amplify DNA tenfold or more is used in conjunction with a similar fraction prepared from cells that do not contain amplified DNA, these kinetically purified sequences can be useful probes with which to screen genomic libraries for amplified DNA (*15*). We have used such probes to successfully screen complementary DNA (cDNA) libraries for transcripts encoded by amplified genes in neuroblastoma cells, and to demonstrate the presence of amplified sequences in genomic DNA by Southern analyses and in cytological preparations by hybridization in situ (*16*).

Double-stranded cDNA was prepared to cytoplasmic poly(A)$^+$ (polyadenylated) RNA from the vincristine-resistant (VCR^r), HSR-containing DC-3F/VCRd-5L cell line and was cloned by poly(dG-dC) tailing into the plasmid vector pUC8. Recombinants (5×10^3) were screened with amplified-DNA probes obtained by C_0t (10–300) enrichment of DNA from DC-3F (VCR^s) or DC-3F/VCRd-5L (VCR^r) cells. Signal intensities from the two screenings were compared and a single clone, designated p5L-18, was found to hybridize with greater relative intensity to the DC-3F/VCRd-5L probe.

Using p5L-18 as the probe, we analyzed Eco RI–digested genomic DNA from DC-3F and DC-3F/VCRd-5L by Southern blots (Fig. 1). Six single-copy bands hybridized in DC-3F; the 4.1 kilobase (kb) fragment was a doublet. Of these six, only three were amplified in DC-3F/VCRd-5L DNA; the other three were present as single copies. Whereas densitometric analyses of these data showed that the gene encoding p5L-18 was amplified approximately 60-fold in the DC-3F/VCRd-5L cell line, the observation that only half of the single-copy bands in DC-3F were amplified was the first indication that multiple genes homologous to the cDNA probe could exist.

To determine if the differential amplification of DNA homologous to p5L-18 occurred in a VCR^r CHL cell line other than DC-3F/VCRd-5L, we analyzed genomic DNA from the independently derived subline DC-3F/VCRm (Table 1). Probes were prepared by digestion of p5L-18 with Pst I and subcloning the resulting 414–base pair (bp) and 88-bp fragments into pUC8. Genomic DNA from the two VCR^r sublines and the parental cell line DC-3F was digested with Eco RI, Bam HI, Hind III, or Pst I, and hybridized with the 414-bp probe (Fig. 2A). Both the p5L-18 probe and the 414-bp probe derived from it hybridized with the same Eco RI fragments in DC-3F and DC-3F/VCRd-5L cells (Fig. 1 and Fig. 2A, lanes a and b). DC-3F/VCRd-5L cells amplified only a subset of the bands that hybridize with the 414-bp probe in DC-3F, but DC-3F/VCRm cells amplified all of those fragments. This differential amplification of DNA fragments was also evident when similar digests were hybridized with the 88-bp probe (Fig. 2B). The results obtained with the 414-bp probe, however, indicated that the 4.1-kb Eco RI fragment amplified in both DC-3F/VCRd-5L and DC-3F/VCRm cells (Fig. 2A, lanes a, b, and c) must be different from the 4.1-kb Eco RI fragment detected by the 88-bp probe, because the 88-bp fragment was amplified only in DC-3F/VCRm cells (Fig. 2B, lanes a, b, and c). Hence the p5L-18 cDNA sequence hybridized to six Eco RI fragments in DC-3F DNA, all of which were amplified in DC-3F/VCRm cells but only three of which were amplified in DC-3F/VCRd-5L cells. Thus, at least two related genes, termed multidrug-resistance associated (MDRA) genes, exist in the DC-3F genome, the amplification of one or all of

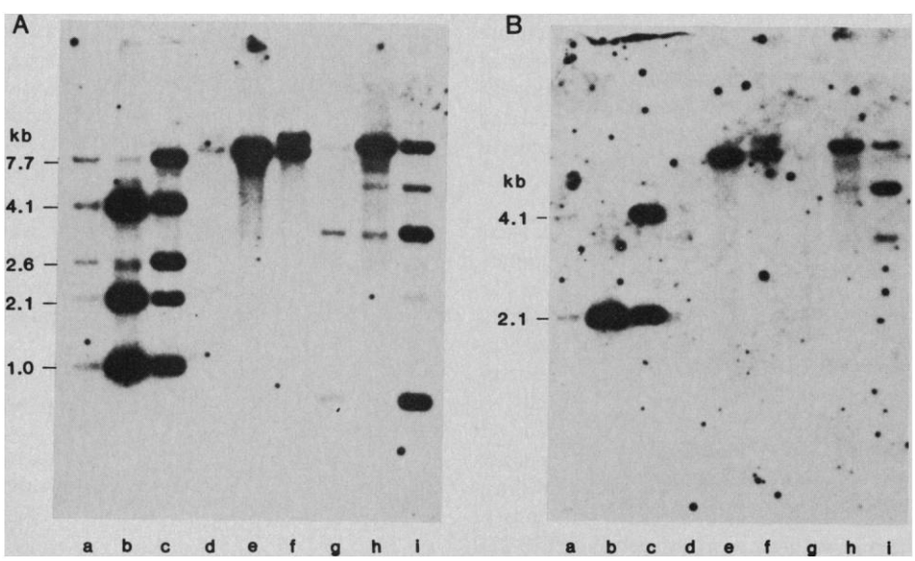

Fig. 2. Southern blot analyses with p5L-18 subclones as probes. Digestion of p5L-18 with Pst I yields two fragments 414 and 88 bp in length. Each fragment was subcloned into the plasmid vector pUC8 and nick-translated to a specific activity of 2×10^8 cpm/μg. Southern blot analyses were performed as described (Fig. 1). Genomic DNA's were digested with either Eco RI (lanes a, b, and c), Bam HI (lanes d, e, and f) or Hind III (lanes g, h, and i). (Lanes a, d, and g) DC-3F DNA, 20 μg; (lanes b, e, and h) DC-3F/VCRd-5L DNA, 10 μg; (lanes c, f, and i) DC-3F/VCRm DNA, 10 μg. (A) Probed with p5L-18-414. (B) Probed with p5L-18-88.

which may be selected for during challenge by vincristine.

To determine whether MDRA genes are amplified in multidrug-resistant cell lines selected for resistance to agents other than vincristine, we analyzed genomic DNA from DC-3F/DMXX (daunorubicin selected), DC-3F/ADX, and DC-3F/ADXC (both actinomycin D selected) cell lines (Table 1). Each of these independently selected cell lines amplified all six Eco RI fragments, as seen in the DC-3F/VCRm cell line (Fig. 3).

Northern blot analyses of poly(A)$^+$ RNA or total cytoplasmic RNA, with p5L-18 as the probe, revealed an increase in two messenger RNA (mRNA) species in DC-3F/VCRd-5L, DC-3F/DMXX, DC-3F/ADX, and DC-3F/ADXC cells (Fig. 4A). The predominant species was 4.5 kb in length, and the minor one was approximately 2.3 kb. Hence, at least one of the MDRA genes was overexpressed in multidrug-resistant CHL cells. Quantitation of RNA levels by dot blot analysis (Fig. 4B and Table 1) suggested that the amount of MDRA gene–specific RNA in DC-3F/VCRd-5L and DC-3F/DMXX cells was less than might be expected from the increase in gene copy number; DC-3F/VCRd-5L amplified one gene about 60 times to yield 60 additional genes, DC-3F/DMXX amplified two genes about 30 times to yield 60 additional genes, while RNA levels are approximately 16- and 22-fold increased, respectively.

In contrast, the steady-state level of MDRA gene RNA in DC-3F/ADX cells, although increased 16-fold over that of DC-3F cells, was accompanied by only a threefold increase in MDRA gene number (Table 1). Indeed, the most dramatic example of the disparity between MDRA gene copy number and RNA level occurred in the DC-3F/ADXC cell line where a fivefold gene amplification was associated with a 97-fold increase in RNA (Table 1). Hence, the steady-state level of MDRA gene RNA in multidrug-resistant Chinese hamster cells is not mediated solely by the amplification of MDRA genes.

Although MDRA gene expression was elevated in each of the multidrug-resistant cell lines tested, we could not establish a clear relation between the amount of gene expression and the amount of resistance or cross-resistance (Table 1). If direct correlation did exist, it was probably between the level of MDRA gene expression and the degree of resistance that each cell line displayed to its selective agent, rather than to the agents to which it was cross-resistant. In addition, in DC-3F/ADXC cells, a 50,000-fold increase in resistance to the selective agent actinomycin D occurred simultaneously with a 97-fold increase in MDRA gene expression, but without parallel increases in cross-resistance (Table 1). Thus, resistance to the selective agent and cross-resistance to other drugs could be uncoupled in CHL cells; this, therefore, shows that increased MDRA gene expression need not directly enhance cross-resistance.

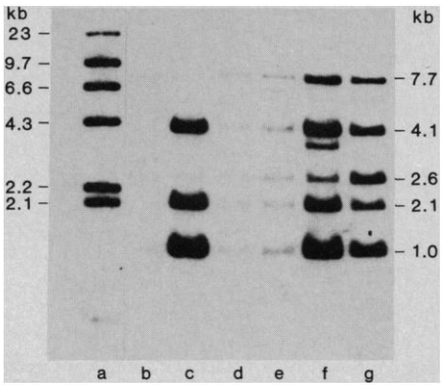

Fig. 3. Southern blot analysis of DNA from cell lines selected for resistance with agents other than vincristine. Hybridization analyses of Eco RI–digested genomic DNA's were performed under conditions described in Fig. 1 with 200 ng of p5L-18 DNA (2×10^8 cpm/μg) as probe. (Lane a) Hind III–digested λ DNA; (lane b) DC-3F DNA, 20 μg; (lane c) DC-3F/VCRd-5L DNA, 10 μg; (lane d) DC-3F/ADX DNA, 20 μg; (lane e) DC-3F/ADXC DNA, 20 μg; (lane f) DC-3F/DMXX DNA, 10 μg; (lane g) DC-3F/VCRm DNA, 10 μg.

Southern blot analysis of VCR^r mouse and human cell lines (Table 1) indicated that all three drug-resistant sublines amplify a gene bearing strong homology to the Chinese hamster MDRA cDNA probe (Fig. 5). MC-IXC/VCR amplified only three of the four Eco RI fragments in the control DNA's (lanes b, c, and e), while SH-SY5Y/VCR amplified only two of these four fragments; this suggested that the multiplicity of MDRA genes observed in CHL cells may also exist in human cells. The presence of a fourth fragment in drug-sensitive cells that was not amplified in either of the drug-resistant sublines suggested that the multiplicity in human cells may involve more than two genes or may include a pseudogene.

Northern blot analysis of MAZ/VCR poly(A)$^+$ RNA confirmed the overexpression in multidrug-resistant mouse cells of an RNA of similar size to that produced in multidrug-resistant CHL cells, while dot blot analysis (Fig. 4B) indicated that overexpression of MDRA genes occurred in multidrug-resistant human cells as well.

We have here identified genes whose amplification and overexpression are closely associated with the multidrug-resistance phenotype in Chinese hamster, mouse, and human cells. Although amplification of

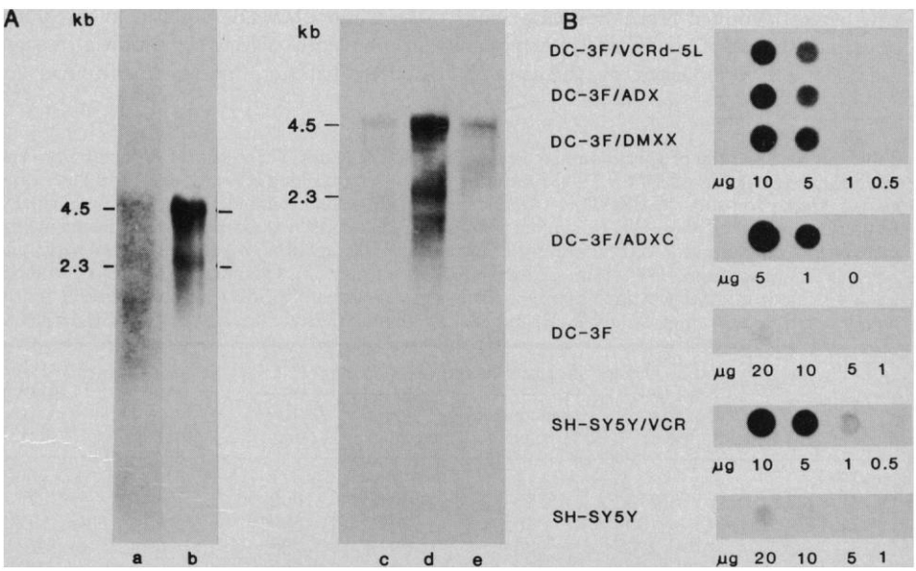

Fig. 4. Northern and dot blot analyses of RNA from sensitive and multidrug-resistant cell lines. Total RNA was isolated from cells by guanidinium isothiocyanate extraction (*25*). Cytoplasmic poly(A)$^+$ RNA was isolated by oligodeoxythymidylate chromatography (*21*). For Northern blot analysis, poly(A)$^+$ RNA or total RNA was denatured and separated by electrophoresis on 1.5% formaldehyde gels, then transferred to nitrocellulose according to Thomas (*26*). Hybridizations were with 200 ng of p5L-18 DNA (1×10^8 cpm/μg) in 50% formamide, 3.5× SSC, and 10% dextran sulfate at 42°C for 16 hours; washings were at 55°C in 0.1× SSC and 0.1% SDS. Dot blot analysis of total RNA was performed as described (*19*). (A) Northern blots. (Lane a) DC-3F poly(A)$^+$ RNA, 5 μg; (lane b) DC-3F/VCRd-5L polyA$^+$ RNA, 5 μg; (lane c) DC-3F/DMXX total RNA, 20 μg; (lane d) DC-3F/ADXC total RNA, 20 μg; (lane e) DC-3F/ADX total RNA, 20 μg. Size estimates were based on migration of 28*S* and 18*S* ribosomal RNA markers. (B) Dot blots. Origin of the RNA and amounts of total RNA per dot are indicated in the figure.

DNA in drug-resistant and multidrug-resistant cells is well documented (*1, 10, 27*), the nature of MDRA gene amplification is unusual because (i) at least two MDRA genes exist in the parental genome and can be amplified together or independently, (ii) mapping of the MDRA genes in DC-3F cells to a single chromosomal site at 1q26 (*28*), together with the observation that within a given subline the amplified MDRA genes are located within the HSR or ABR (*28*), indicate that this differential amplification most likely occurs from a single locus, (iii) the level of expression of the MDRA genes can far exceed the level of their amplification and therefore is not due solely to gene dosage, and (iv) the level of MDRA gene expression, while possibly correlated with the degree of resistance to the selective agent, does not correlate with the levels of crossresistance. These observations are of particular significance in view of the fact that the p5L-18 cDNA clone is strongly homologous to p-glycoprotein cDNA (*29*). Hence, at least one of the MDRA genes reported here is related to and may share identity with the p-glycoprotein genes known to be amplified and overexpressed in colchicine selected multidrug-resistant CHO cells and multidrug-resistant mouse and human cells as well (*10*).

Thus we have shown differential amplification of related genes from a single locus and, in the case of the MDRA (p-glycoprotein) genes identified here, these may have potential functional significance. Such significance is not indicated by the data in Table 1, however, since no striking difference in resistance or multidrug-resistance pattern is observed in the CHL cell lines, regardless of the number of MDRA genes amplified. Whether more than one of the genes identified here is actually functional is unknown.

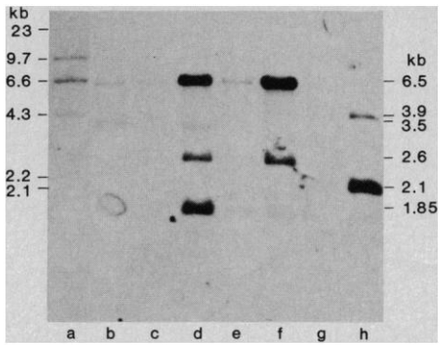

Fig. 5. Southern hybridization analysis of genomic DNA from mouse and human multidrug-resistant cells. Hybridizations were performed under the stringent conditions described in Fig. 1, with 200 ng ^{32}P-labeled p5L-18 as probe. 10 μg of each DNA was digested with Eco RI before electrophoresis. An overexposure of these filters indicated the presence of each of the bands in the control DNA's. (Lane a) Hind III–digested λ DNA marker; (lane b) human placental DNA; (lane c) MC-IXC DNA; (lane d) MC-IXC/VCR DNA; (lane e) SH-SY5Y DNA; (lane f) SH-SY5Y/VCR DNA; (lane g) MAZ DNA; (lane h) MAZ/VCR DNA.

Although the amplification and overexpression of MDRA genes in all of the independently selected multidrug-resistant Chinese hamster, mouse, and human cell lines establishes their association with the multidrug-resistant phenotype, the functional significance of this association remains unclear. If MDRA, that is, the p-glycoprotein, gene expression is directly responsible for the establishment of resistance to the selective agents, then one would expect the level of expression to be positively correlated with the degree of resistance to those agents. Such a correlation exists in the vincristine- and actinomycin D–selected cell lines and, to a lesser extent, in the daunomycin-selected line (Table 1). Thus, to the extent that resistance levels measured by ED_{50} values are an accurate reflection of resistance mechanisms, the MDRA gene product may be involved in and be directly responsible for resistance to the selective agent. It seems less likely that multidrug resistance also may be directly mediated by MDRA gene expression because there is no correlation between the degree of cross-resistance and the level of MDRA gene expression (Table 1). Hence, the establishment of resistance and the establishment of cross-resistance are distinct phenomena. In fact, in multidrug-resistant cells, resistance is almost always greatest to the drug used for the initial challenge. In addition, although the transport of vincristine in DC-3F/VCRd-5L cells is altered sufficiently to account for resistance (*30*), very little change in the transport of daunomycin occurs in these cells; this suggests that the development of cross-resistance to this agent requires an additional mechanism.

Alternatively, the overexpression of MDRA genes in multidrug-resistant cells may be a secondary event that occurs as a result of the establishment of multidrug resistance and the overexpression of MDRA genes would be one of the effects, rather than the cause, of resistance and multidrug resistance. Thus, overexpression of MDRA genes may fulfill a requirement for cell survival during maintenance of the multidrug-resistant phenotype. Further clarification of the nature and function of the MDRA gene product or products will distinguish between these possibilities.

Table 1. Characteristics of sensitive and multidrug-resistant cell lines. The values for drug resistance and multidrug resistance listed for the DC-3F series of Chinese hamster lung cells are taken from Peterson *et al.* (*6*) except for the DC-3F/ADXC subline, where the values were determined experimentally. Establishment of the DC-3F/ADXC subline from DC-3F/ADX cells was accomplished by increasing the maintenance concentration of actinomycin D from 10 to 100 μg/ml (*2, 6*). Values for the MAZ and SH-SY5Y lines are from (*14*), and those for MC-IXC are from (*17*). Quantitation of DNA and RNA levels was by densitometric scanning of x-ray films with a Beckman DU-8B spectrophotometer and by liquid scintillation counting of DNA (*18*) and RNA (*19*) dot blots hybridized with [^{32}P]p5L-18 DNA.

Cell line	Degree of resistance and cross-resistance*				MDRA gene copy number†	Level of MDRA expression‡
	Actinomycin D	Daunorubicin	Vincristine	Colchicine		
DC-3F	1	1	1	1	2	1
DC-3F/VCRd-5L	270	180	2800*	1000	60	16
DC-3F/VCRm	40	50	600*	600	50	ND
DC-3F/DMXX	270	900*	300	700	60	22
DC-3F/ADX	2500*	50	1400	300	6	16
DC-3F/ADXC	50,000*	130	1900	700	10	97
MAZ			1		1	ND
MAZ/VCR			4000*		~50	ND
SH-SY5Y	1		1		2	1
SH-SY5Y/VCR	120		1300*		~50	90
MC-IXC	1		1		2	ND
MC-IXC/VCR	450		6600*		~50	ND

*Indicates the selective agent. †On the assumption that two nonallelic genes are present in DC-3F. ‡On the assumption that DC-3F cells produce 1 unit of RNA. ND, not determined.

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29. We are indebted to P. Borst and colleagues for providing p-glycoprotein cDNA clone cp28 (A. M. Van der Blick, T. Van der Velde-Koerts, V. Ling, P. Borst, *Mol. Cell. Biol.*, in press) with which our cDNA clone p5L-18 cross-hybridizes under normal stringency conditions. Clone cp28 in turn cross-hybridizes with the p-glycoprotein encoding cDNA pCHP1 that was isolated from a λgt11 expression library with a p-glycoprotein specific monoclonal antibody (*10*).
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Activation of the AIDS Retrovirus Promoter by the Cellular Transcription Factor, Sp1

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The nature and position of transcriptional control elements responsible for the expression of genes encoded by the retrovirus associated with acquired immune deficiency syndrome (AIDS) have not been precisely defined. In this study it is shown that the mammalian Sp1 transcription factor binds to promoter sequences within the AIDS retrovirus long terminal repeat (LTR) and activates RNA synthesis five- to eightfold in reconstituted reactions in vitro. Experiments in which regions of DNA were protected from added reagents by specifically bound proteins (footprinting) indicated that the upstream promoter region of the AIDS virus LTR lies between −45 and −77 (relative to the RNA start site, +1) and contains three tandem, closely spaced Sp1 binding sites of variable affinity. Base-substitution mutations targeted to one or all three Sp1 binding sites were found both to eliminate the binding of Sp1 and to cause up to a tenfold reduction in transcriptional efficiency in vitro. These findings suggest that one important component of the AIDS virus transcriptional control region interacts with a cellular transcription factor, Sp1, and that this factor must function in conjunction with transcriptional elements located downstream of the RNA cap site to mediate the response of the LTR to viral *trans*-activation.

TRANSCRIPTION OF RETROVIRAL GENOMES is mediated by promoter elements that are located within the viral long terminal repeat (LTR) segments, and can be strongly regulated by viral *trans*-activator proteins. Because these elements and those of other viruses of eukaryotic cells rely on the cellular RNA polymerase II transcriptional apparatus, they are considered excellent models for analysis of the mechanism by which RNA synthesis is initiated and regulated. A combined genetic and biochemical approach to the study of promoter recognition has proved particularly useful in defining the role of specific RNA polymerase II transcription elements. For example, it has recently been shown that certain viral and cellular genes bind a cellular transcription factor designated Sp1, and these specific protein–DNA interactions contribute directly to promoter strength in vitro (*1–4*) as well as in vivo (*5, 6*). These studies have further revealed the following biochemical properties of Sp1 (*7*): (i) A promoter of Sp1 recognizes an asymmetric decanucleotide sequence (G/T GGGCGG-PuPuPy) with an affinity determined by the match of a given sequence to the consensus. (ii) A single Sp1 binding site is sufficient to activate transcription, and both binding and transcriptional activation are independent of the orientation of the binding site relative to the RNA start site. (iii) Sp1-responsive promoters generally contain multiple Sp1 binding sites located within 40 to 150 bp upstream of the RNA start site, and Sp1 binding sites are sometimes intermingled with those for other promoter-specific transcription factors. In the latter case, the coordinate interaction of two or more different factors may be required for transcriptional activity of the promoter (*3*). (iv) Weaker binding sites may be positioned at optimal locations adjacent to the TATA box, and these may be equivalent to or even more important for promoter strength than higher affinity Sp1 sites located at more distal positions. (v) Genes that are recognized by Sp1 are expressed or regulated in vivo by a variety of distinct mechanisms, including enhancer activation [for example, the SV40 early promoter (*5, 8*)], viral *trans*-activation of promoter elements [for example, the thymidine kinase gene of herpes simplex virus (HSV) (*9*)], and induction through upstream response elements [for example, the HSV immediate-early and human metallothionein genes (*10*)]. Here we report that Sp1 is also an important component of the promoter for a lymphocytopathic RNA virus, the retrovirus [variously termed ARV-2, LAV, or HTLV-III (*11, 12*)] associated with the acquired immune deficiency syndrome (AIDS) and related syndromes (*13, 14*).

Promoter and enhancer elements within the LTR segments of retroviruses characteristically generate transcripts of full genomic length that may be differentially spliced to yield a variety of mRNA species (*15*). Inspection of the AIDS retrovirus LTR re-

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