Transcription and Processing of RNA from Mouse Ribosomal DNA Transfected into Hamster Cells

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Transcription of mouse genes coding for rRNA in CHO cells was promoter dependent at levels 3 to 10% of that of endogenous rRNA synthesis. Northern (RNA) and S1 nuclease mapping analyses demonstrated that transcription proceeds through the entire gene segment coding for rRNA in transfected constructs and continues, at least in some cases, into the adjoining plasmid sequences. S1 nuclease mapping also detected some processing cleavages in the transcripts, including those at the 3' terminus of 18S rRNA, those at the rapidly cleaved site at +650 in the external transcribed spacer, and those at a previously uncharacterized, rapidly cleaved site in the internal transcribed spacer. Deletion of sequences upstream or downstream from the promoter generally had no measurable effect on the level of transcription, but deletion of a 300-base-pair *XhoI-XhoI* fragment starting 1,287 base pairs from the transcription start site sharply increased the steady-state level of rRNA. Effects on processing were harder to test, because many intermediates are too unstable to detect even by S1 nuclease mapping; however, the data suggest that RNAs with deletions in the external transcribed spacer are processed poorly at distal sites. Processing at some sites may thus depend on interactions involving distant segments of rRNA.

The processing of mRNA precursors has been extensively analyzed in extracts and in cells (16, 18). In contrast, comparable information on the processing of rRNA remains sparse. While it is known that rRNA processing in mammalian cells proceeds through a set of simple cleavages, no in vitro system which produces fully processed rRNAs has yet been devised. Even in vivo, the expression of transfected constructs of genes coding for rRNA (rDNA) is difficult to study because of the very high background of endogenous rRNA formation. One way to overcome this problem is to analyze the expression of transfected heterologous rDNA with probes unique to spacer sequences in the transfected rDNA (9). Even though rDNA transcription has been shown to be restricted by species-specific barriers (10, 14), it can be studied in cells or extracts of some closely related species (human and monkey [12], mouse and rat [20], and mouse and hamster [7]). In those cases, the spacer sequences are different enough to permit adequate discrimination of transcripts from endogenous and transfected rDNA. We have used this approach to analyze features of the transient expression of tracts of rDNA transfected into culture cells.

MATERIALS AND METHODS

Cell culture. Mouse L cells and Chinese hamster ovary (CHO) cells were grown in minimal essential medium supplemented with 10% fetal calf serum.

Recombinant rDNA plasmids. Figure 1 shows a schematic map of the subclones and deletion constructs used in the transient expression experiments. All plasmids used for transfection were cloned in the vector pUC18. Mouse clone numbers are as described elsewhere (2, 3). pCL3 was derived by subcloning the 3.2-kilobase (kb) *SalI-SalI* fragment of pMr974 (20), and it contained the mouse rDNA promoter and 3.0 kb of downstream DNA. pM18 was constructed by ligating the 13-kb pMr974 *EcoRI-EcoRI*

insert into the *EcoRI* site of clone 6 in the appropriate orientation. The resulting construct, containing the mouse rDNA promoter, 8 kb of upstream DNA, and an intact transcription unit through the first one-third of internal transcribed spacer 2 (ITS2), was the parent molecule for a number of constructs. pRD18 contained a 1.2-kb deletion in the region upstream of the start site; it arose spontaneously in a set of repeated sequences (the exact endpoints are unknown); pBL18 contained a 5.5-kb *BalI-BalI* deletion in the clone 1 region of pM18. This deletion removed certain restriction sites, facilitating further constructions starting from pBL18. By using standard recombinant DNA technology (i.e., filling in of restriction sites with the Klenow fragment of DNA polymerase I followed by blunt-end ligation), the following deletions were made: ΔETS1, a *ScaI-XhoI* deletion that removed nucleotides +463 to +1587, including the +650 processing site; ΔETS2, an *XhoI-XhoI* deletion from +1287 to +1587; ΔETS3, an *XhoI-TthIII1* deletion from +1287 to +3831; ΔETS4, a *ScaI-TthIII1* deletion of most of the external transcribed spacer (ETS) region (nucleotides +463 to +3831); and ΔETS5, a *TthIII1-BamHI* deletion that removed 165 base pairs (bp) of ETS adjacent to the 18S coding region and 590 bp of the 5' 18S sequence.

Transfections. rDNA clones were introduced into cells by the calcium phosphate method of Wigler et al. (21). The most reproducible results were obtained with DNA purified twice by centrifugation to equilibrium in CsCl gradients. The calcium phosphate-DNA precipitate was allowed to form for 15 min and then added for 15 min to 10⁶ cells stripped of growth medium (pregrown in 10-cm-diameter petri dishes in fresh medium supplemented with 10% fetal calf serum). Fresh medium was then added, and after 15 h the medium was replaced and the incubation was continued for another 25 h. Cells were then harvested, and RNA was extracted.

RNA preparation. RNA was extracted by the guanidinium-CsCl method as described by Maniatis et al. (13). Before analysis, the RNA preparations were digested with 0.3 U of RNase-free DNase I (Pharmacia) per μg of RNA for 30 min

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FIG. 1. Schematic of mouse rDNA subclones and deletion constructs. The names of constructs used in transfection experiments are shown at the left. The map at the top is a schematic of mouse rDNA including the transcription initiation site ($\rightarrow$) and the 18S, 5.8S, and 28S coding regions (■). Numbers below the map refer to previously described subclones (3). The expanded section shows the extent of mouse rDNA used in constructing the subclones and deletions and indicates the transcription initiation site ($\rightarrow$) and processing sites ($\downarrow$) examined in this study. The constructs shown below the expanded map were used in transfections. The endpoints of the clones and the restriction sites used to generate deletions are indicated. Restriction endonuclease abbreviations: E, *EcoRI*; K, *KpnI*; S, *SalI*; B1, *BalI*; P, *PvuII*; Sc, *Scal*; X, *XhoI*; T, *TthIII*; B, *BamHI*; H, *HindIII*; A, *AclI*.

at 25°C. The RNA preparations were stored precipitated in ethanol.

S1 nuclease analysis. S1 nuclease mapping experiments were done as described elsewhere (1). Probes were prepared by digesting DNA with the appropriate restriction enzyme and then labeling the DNA at the 5' terminus with polynucleotide kinase or at the 3' terminus with the Klenow fragment (13). The labeled DNA was then digested with a second enzyme, and the probe was purified by electrophoresis in polyacrylamide gels.

Northern (RNA) blot analyses. RNA was dissolved in 50% formamide–2.2 M formaldehyde–MOPS buffer {20 mM MOPS [3-(*N*-morpholino)propanesulfonic acid], 5 mM sodium acetate, 1 mM EDTA [pH 7.0]}. Samples were heated for 5 min at 65°C to denature the RNA and then loaded onto 0.9 to 1.0% agarose gels containing 2.2 M formaldehyde in MOPS buffer. Electrophoresis was performed for 5 h at 80 V. A mixture of RNAs (0.3 to 9.5 kb; Bethesda Research Laboratories, Inc.) provided size markers. The gel containing the fractionated RNA was rinsed in 20× SSC (1× SSC is 150 mM NaCl plus 15 mM sodium citrate), and the RNA was transferred to a nitrocellulose filter (19). The filter was prehybridized in 2× SSC–0.02% bovine serum albumin–0.02% Ficoll–0.02% polyvinylpyrrolidone–0.1% sodium dodecyl sulfate–500 μg of sonicated, denatured calf thymus DNA per ml for 4 h at 68°C. The solution was replaced with another portion containing nick-translated (17) or random-primered (8) 32 P-labeled probe. Hybridization was performed for 18 h at 68°C. Filters were then washed twice for 30 min in 2× SSC and twice more in 0.1× SSC–0.1% sodium dodecyl sulfate at 65°C. Autoradiography was done with Kodak XAR film with intensifying screens.

FIG. 2. Transcription of mouse rDNA constructs transfected into hamster cells, with S1 nuclease analysis of the transcription start sequence. The probe (P) was a *SalI*–*PvuII* fragment 5' end labeled at the *PvuII* site (*). The hybridization temperature was 49°C, and the S1 digestion temperature was 30°C. Lane M, Molecular size markers (sizes in base pairs); lane 1, probe plus carrier tRNA; lane 2, 0.2 μg of L-cell RNA; lane 3, 10 μg of CHO RNA; lanes 4 to 8, 10 μg of RNA from cells transfected with pM18, pMr974, pΔETS5, pRD18, and pC13, respectively. At the bottom the probe (P) and the protected-fragment (A) sizes are illustrated schematically. Tx, Start site of transcription.

RESULTS

Levels and sizes of rRNA transcripts from transfected DNA constructs. Because the sequences of mature rRNAs are almost identical in the species studied, transcription was detected with probes unique to spacer sequences. As expected, transcription from the normal start site on transfecting mouse rDNA was seen in CHO cells. In general, the level of transcription from transfected rDNA was 3 to 10% of that from endogenous DNA. (For example, compare the level of transcription from transfected rDNA with the signal from increasing amounts of endogenous mouse RNA [see Fig. 3].) The steady-state level of transcripts of specific regions of ETS, analyzed by S1 nuclease mapping, was generally lower from DNAs with shorter tracts of the transcription unit. Most marked was the consistently lower level of transcription when the transfected DNA contained only the promoter and 3.0 kb of ETS (Fig. 2, lanes 4 to 7 versus lane 8 [pC13]). The expression for a construct ending within the 18S rRNA (pMr974) was also somewhat lower than that for one that continued through the ITS1 and 5.8S rDNA into ITS2.

Upstream sequences were also tested for possible direct effects on transcription. Studies of rat rDNA transcription *in vitro* have indicated that some sequence between –286 and –1,018 nucleotides upstream from the initiation sequence enhances transcription (6). Another upstream region in rat, mouse, and human rDNAs contains stretches of alternating purine and pyrimidine (4), which have been suggested to be components of some enhancers (15). No difference in the level of the transient expression was seen between intact rDNA and constructs lacking the poly(GT) region or most of the region of repeated sequences just upstream from the promoter (data not shown). Most deletions inside the ETS region of the transcription unit also had little effect on the observed level of expression. Deletion of the 5' sequence of 18S rRNA (ΔETS5), for example, had little effect on the level of expression observed (Fig. 2, lane 6) compared with the level seen with undeleted DNA (pM18). In one case,

FIG. 3. Northern analysis of rRNA transcripts containing ETS and ITS1 sequences of transfected mouse rDNA. Total RNA was transferred from a 0.9% agarose gel to a nitrocellulose filter and hybridized with a random-primed probe, which was either a 300-bp *XhoI-XhoI* fragment (A) or a 228-bp *AccI-KpnI* fragment (B). Sizes are in kilobases. (A) Lanes 1 to 3, Decreasing amounts of RNA (3, 0.9, and 0.3 μ g, respectively) from nontransfected L cells; lane 4, 10 μ g of CHO cellular RNA; lanes 5 to 8, 10 μ g of RNA from CHO cells transfected with pM18, Δ ETS2, pRD18, and pMr974, respectively. The film was exposed for 2 h at -80°C with intensifying screens. (B) Lanes 1 and 2, L-cell RNA (1 and 0.3 μ g of total RNA, respectively); lane 3, 10 μ g of CHO RNA; lanes 4 to 6, 10 μ g of RNA from CHO cells transfected with pM18, pBL18, and Δ ETS2, respectively. Exposure was for 4 days at -80°C with intensifying screens. Lane 6B, 16-h exposure of the RNA represented in lane 6.

however, when a relatively small (300-nucleotide) deletion was introduced between two *XhoI* sites in the ETS (Fig. 1, Δ ETS2), the resultant level of expression was consistently much higher than that in the control and in other cases studied (e.g., see Fig. 3B, lane 6). It is conceivable that the small *XhoI-XhoI* deletion eliminated a site at which RNA polymerase movement is ordinarily slowed drastically to limit the rate of rRNA production, but it seems more likely that the RNA with a deleted segment represented a longer, more stable transcript.

The notion that nascent transcripts reach considerable length was tested directly by Northern analysis. In Fig. 3A, the sizes of the various rRNA species observed in endogenous mouse cell RNA are compared with those of RNA from hamster cells transfected with several mouse rDNA constructs. The probe was a 300-bp *XhoI-XhoI* fragment of the ETS. As in the S1 nuclease mapping assays of the start site for transcription (Fig. 2), nontransfected hamster cell RNA gave no signal detectable over background (Fig. 3A, lane 4), and transfected rDNA was clearly expressed. The mouse-specific rRNA bands included a major species at 6.1 kb in every case. That is the size expected for a transcript extending from the +650 processing site to a site in the neighborhood of the 5' end of 5.8S rRNA (34S in references 2 and 3) at the end of the ITS1 region. The 6.1-kb species indeed contained ITS1 sequences: Fig. 3B, for example, shows the expected positive signals with a 228-bp ITS1 probe from total mouse RNA (lanes 1 and 2) that were absent in total hamster RNA (lane 3). The prominent 6.1-kb band was again seen in the transfected cellular RNA (lanes 4 to 6). Because the level of expression of the Δ ETS2 construct was so high, sixfold-lower exposure time was required to visualize the 6.1-kb transcribed species by autoradiography (Fig. 3B, lane 6B). No vector-specific sequences were detected in RNA from the transfected cells, except for the two constructs in

which the rDNA insert contained less than the full 18S rRNA sequences (Fig. 1, pMr974 and PCL3). In those instances, long transcripts bearing pBR322 sequences were consistently detected (data not shown). Transcription may continue into vector sequences in all cases, but when the 18S rDNA sequence is present intact, cleavages that yield the 6.1-kb species probably expose distal RNA sequences to rapid degradation.

Processing of transcripts from transfected rDNA. As in previous studies, the 5'-terminal fragment produced by rapid cleavage at nucleotide +650 in mouse rRNA (and in other studies, at +414 in human rRNA [11]) was especially easily detected. Figure 4 illustrates the cleavage in mouse RNA. The amount of +650 fragment detected by S1 nuclease mapping in steady-state RNA was proportional to the level of transcription of the rDNA construct (these were the same transfection experiments as those assessed for accurate transcription initiation [Fig. 2]; here, conditions were optimized to detect the cleavage fragment rather than the start site). As with transcription, the presence or absence of most of a region upstream from the transcription unit had no effect on the production of the +650 terminus (Fig. 4, lane 7 versus lane 4). Similarly, as expected, cleavage occurred even when the 5' portion of 18S was deleted (lane 6 [Δ ETS5]). Much less steady-state RNA or fragment production was observed, however, when the transcribed rRNA was limited to a small portion of the ETS (lane 8 [pCL3]).

To assess the formation of the 3' terminus of 18S rRNA, we used a probe covering the 3' end of 18S rRNA and much of the ITS1 sequence. Figure 5 shows the two characteristic signals observed both in endogenous mouse RNA and in RNA from transfected cells. One RNA fragment starts at the 5' end of ITS1, abutting the 3' end of 18S rRNA; the other starts at a previously uninvestigated rapid-cleavage site in the ITS1 sequence (Fig. 5A, bottom). The latter fragment

FIG. 4. Processing of rRNA transcripts from transfected mouse rDNA at the +650 nucleotide site. Mouse rDNA constructs were transfected into CHO cells, and the processing cleavage at +650 was assayed by using S1 nuclease. The probe (P) was a *SalI*-*XhoI* fragment 5' end labeled at the *XhoI* site (*). The hybridization temperature was 65°C, and the S1 digestion temperature was 22°C. Lane M, Molecular size markers (sizes in base pairs); lane 1, probe plus carrier tRNA; lane 2, 0.5 µg of L-cell RNA; lane 3, 10 µg of CHO RNA; lanes 4 to 8, 10 µg of RNA from CHO cells transfected with pM18, pMr974, pΔETS5, pRD18, and pCL3, respectively. The start site of transcription (Tx) (→) and the +650 cleavage site (↓) are indicated in the map at the bottom, as are the probe (P) and protected-fragment (A) sizes.

was seen in RNA transcribed from constructs pRD18 and pM18, both of which extended through the ITS1 sequence (Fig. 5A, lanes 4 and 5). From a number of S1 nuclease experiments with probes like those represented in Fig. 5, it was determined that the cleavage takes place about 55 nucleotides distal to the 3' terminus of 18S rRNA in mouse ITS1. The precise cleavage point has not been confirmed, but is probably at the 3' end of a sequence that includes a series of pyrimidines followed by (GU)₄.

Since the two cleavages near the 3' end of 18S rRNA produced discrete fragments, one could ask whether similar processing events are detected in constructs lacking various parts of upstream rDNA. The fragment arising from the cleavage at the 3' end of 18S rRNA was at the limit of sensitivity of the method, but for the species produced by cleavage in the ITS1, the result seems clear: when portions of the 5' ETS RNA or upstream 18S rRNA were deleted, no signal arising from the cleavage in ITS1 was detected (Fig. 5B, lanes 6 to 10). Cleavage failed even with deletion construct ΔETS2, which produced very large amounts of transcript.

DISCUSSION

The expression of transfected rDNA was high enough to permit analysis of transcripts and some processing events, but the experiments were at the limit of resolution of the techniques. Here we discuss the results and some of the limitations.

Mouse rRNA transcripts were formed in transfected cells at a level 3 to 10% of that of endogenous rDNA synthesis. Transcription probably continues into vector sequences, but

FIG. 5. Processing of rRNA transcripts from transfected mouse rDNA at the 3' terminus of 18S rRNA. (A) Formation of the 3' 18S terminus was assayed on total RNA from transfected CHO cells. The probe (P) was a *HindIII*-*AccI* fragment end labeled at the *AccI* site (*) and spanned the 18S-ITS1 junction. Hybridization was done at 65°C, and S1 digestion was done at 30°C. Lane M, Molecular size markers (sizes in base pairs); lane 1, probe plus carrier tRNA; lane 2, 0.5 µg of L-cell RNA; lane 3, 10 µg of CHO RNA; lanes 4 and 5, 10 µg of RNA from CHO cells transfected with pM18 and pRD18, respectively. The map illustrates the probe (P) and the two cleavages detected (↓ and A and B). (B) Mouse rDNA deletion constructs assayed for processing at the 3' terminus of 18S RNA by using the same probe. Lane M, Molecular size markers (sizes in base pairs); lane 1, probe plus carrier tRNA; lane 2, 0.5 µg of L-cell RNA; lane 3, 10 µg of CHO RNA; lanes 4 to 10, 10 µg of RNA from CHO cells transfected with pM18, pBL18, pΔETS1, pΔETS2, pΔETS3, pΔETS4, and pΔETS5, respectively.

the vector sequences tend to decay too rapidly to be detected.

When the transfected rDNA contained deletions, there was usually little or no effect on the level of transcription. In one case, however, a small deletion in the external spacer (Δ ETS2) sharply increased the steady-state levels of transcribed rRNA. Such an effect on RNA levels is puzzling and may be understood only when much more is known about the conformation and stability of pre-rRNA.

Cleavage sites were inferred primarily from the S1 nuclease results. The results were weakened because many of the spacer rRNA fragments released degraded too quickly *in vivo* to be detected. Nevertheless, rRNA molecules which contained the ETS and most or all of 18S rDNA could be observed to incur the expected cleavages that produce the 5' +650 nucleotide terminus as well as mature 3' ends (and in other experiments, 5' ends [20; unpublished results]) of 18S rRNA. A previously unreported rapid cleavage in ITS1, partially documented here, also occurred in both endogenous mouse rRNA and in transcripts from the transfected rDNA. Finally, the Northern analyses showed a characteristic band (34S rRNA) produced from such constructs, suggesting that another normal cleavage, in the neighborhood of 5.8S rRNA, also occurred.

One rapid cleavage (at nucleotide 650 of the ETS) is known to occur only with local sequence cues and was, as expected, not changed by distant deletions, but the spacer cleavage in ITS1 seemed to be precluded by deletions in the ETS far upstream. This might imply that distant parts of the RNA interact spatially or functionally in nucleoli during processing. However, the levels of the relevant precursor species are so low that the data are only suggestive.

Higher levels of expression of transfected rDNA could be expected if complete units of rDNA were available as the starting material. This would also permit positive controls to show that complete units of rDNA are properly transcribed and processed. Such experiments have been impossible because the complete rDNA unit of 44 kb is too large to be cloned in standard vectors, but in work in progress we have made clones that contain one or more complete tandem repeats of human rDNA units in yeast cell artificial chromosome vectors (5), permitting the initiation of this approach.

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LITERATURE CITED

1. Berk, A. J., and P. A. Sharp. 1981. Sizing and mapping early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. *Cell* 12:721-732.
2. Bowman, L. H., W. E. Goldman, G. I. Goldberg, M. B. Hebert, and D. Schlessinger. 1983. Location of the initial cleavage sites in mouse pre-rRNA. *Mol. Cell. Biol.* 3:1501-1510.
3. Bowman, L. H., B. Rabin, and D. Schlessinger. 1981. Multiple ribosomal RNA cleavage pathways in mammalian cells. *Nucleic Acids Res.* 9:4951-4966.
4. Braaten, D. C., J. R. Thomas, R. D. Little, K. R. Dickson, I. Goldberg, D. Schlessinger, A. Ciccodicola, and M. D'Urso. 1988. Locations and contexts of sequences that hybridize to poly(dG-dT) · (dC-dA) in mammalian ribosomal DNAs and two X-linked genes. *Nucleic Acids Res.* 16:865-881.
5. Burke, D. T., G. T. Carle, and M. V. Olson. 1987. Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. *Science* 236:806-836.
6. Cassidy, B. G., H.-F. Yang-Yen, and L. I. Rothblum. 1986. Transcriptional role for the nontranscribed spacer of rat ribosomal DNA. *Mol. Cell. Biol.* 6:2766-2773.
7. Dhar, V. N., D. A. Miller, and O. J. Miller. 1985. Transcription of mouse rDNA and associated formation of the nucleolus organizer region after gene transfer and amplification in Chinese hamster cells. *Mol. Cell. Biol.* 5:2943-2950.
8. Feinberg, A., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6-13.
9. Goldman, W. E., G. Goldberg, L. H. Bowman, D. Steinmetz, and D. Schlessinger. 1983. Mouse rDNA: sequences and evolutionary analysis of spacer and mature RNA regions. *Mol. Cell. Biol.* 3:1488-1500.
10. Grummt, I., E. Roth, and M. R. Paule. 1982. Ribosomal RNA transcription *in vitro* is species specific. *Nature (London)* 296:173-174.
11. Kass, S., N. Craig, and B. Sollner-Webb. 1987. Primary processing of mammalian rRNA involves two adjacent cleavages and is not species specific. *Mol. Cell. Biol.* 7:2891-2898.
12. Learned, R. M., and R. Tjian. 1982. *In vitro* transcription of human ribosomal RNA genes by RNA polymerase I. *J. Mol. Appl. Gen.* 1:575-584.
13. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
14. Miesfeld, R., and N. Arnheim. 1984. Species-specific rDNA transcription is due to promoter-specific binding factors. *Mol. Cell. Biol.* 4:221-227.
15. Nordheim, A., and A. Rich. 1983. Negatively supercoiled simian virus 40 DNA contains Z-DNA segments within transcriptional enhancer sequences. *Nature (London)* 303:674-679.
16. Padgett, R. A., P. J. Grabowski, M. M. Konarska, S. Seiler, and P. A. Sharp. 1986. Splicing of messenger RNA precursors. *Annu. Rev. Biochem.* 55:1119-1150.
17. Rigby, P. W. J., M. Dieckman, C. Rhodes, and P. Berg. 1977. Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* 113:237-251.
18. Sharp, P. A. 1987. Splicing of messenger RNA precursors. *Science* 235:766-771.
19. Thomas, P. S. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA* 77:5201-5205.
20. Vance, V. B., E. A. Thompson, and L. H. Bowman. 1985. Transfection of mouse rDNA into rat cells: faithful transcription and processing. *Nucleic Acids Res.* 13:7499-7513.
21. Wigler, M., A. Pellicer, S. Silverstein, R. Axel, G. Urlaub, and L. A. Chasin. 1979. DNA-mediated transfer of the adenine phosphoribosyl-transferase locus into mammalian cells. *Proc. Natl. Acad. Sci. USA* 77:1373-1376.