Adenovirus Type 12 E1A Down Regulates Expression of a Transgene under Control of a Major Histocompatibility Complex Class I Promoter: Evidence for Transcriptional Control

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The E1 region of human adenovirus type 12 (Ad12 E1) represses the expression of major histocompatibility complex (MHC) class I genes in transformed primary rodent cells. Conflicting results have been reported as to whether this E1A-mediated repression occurs at a transcriptional or a posttranscriptional level. In the present study, we show that in Ad12 E1-transformed primary baby mouse kidney cells from transgenic mice harboring the human growth hormone gene under control of an H-2K promoter both the expression of the endogenous MHC class I genes and the expression of the transgene are repressed. This experiment, as well as nuclear run-on analyses performed with single-stranded probes, revealed that Ad12 E1A inhibits MHC class I gene expression by repressing its promoter.

Primary rodent cells transformed by the E1 region of adenovirus type 12 (Ad12) show a reduced expression of the major histocompatibility complex (MHC) class I genes (24). Down regulation takes place at the level of mRNA accumulation and is mediated by the 13S-encoded (266-amino-acid) product of the Ad12 E1A region (6, 28). This protein has the ability to *trans*-activate and *trans*-repress a variety of viral and cellular genes at the transcriptional level (for reviews, see references 4 and 14), suggesting that the repression of MHC class I genes also occurs at the level of transcription. However, we were unable to find differences in transcription of MHC class I genes between untransformed, Ad5 E1-transformed, and Ad12 E1-transformed cells by nuclear run-on analysis, indicating that E1A affects MHC class I expression posttranscriptionally (29). This conclusion was in agreement with results obtained by Vogel et al. (32). Using an H-2Ld promoter linked to the bacterial gene for chloramphenicol acetyltransferase, they found no difference in expression level of this construct in Ad5 and Ad12 E1-transformed cells in a transient expression assay. In contrast to these results, Kimura et al. (15) showed that in transient expression assays the H-2Kb promoter is repressed 8- to 10-fold in the presence of Ad12 E1 compared with Ad5 E1, suggesting that the repression of MHC class I genes by Ad12 E1A is regulated transcriptionally. Furthermore, recently both Friedman and Ricciardi (9) and Ackrill and Blair (1), who used nuclear run-on analyses, reported that the regulation of MHC class I gene expression by Ad12 E1A occurs mainly at the transcriptional level.

Nuclear run-on analysis. These conflicting results prompted us to reconsider our previous conclusions based on nuclear run-on assays. Since the MHC class I genes are probably directed by rather weak promoters, a high labeling efficiency of the nascent RNA is required to obtain sufficient hybridization signals. Therefore, we have modified the run-on procedure described previously (22, 29) at some points. Cells were subjected to homogenization in a buffer contain-

ing 50 mM KCl instead of 150 mM KCl; this was followed by two washes of the nuclear pellet with 50 and 150 mM KCl. These modifications resulted in very efficient labeling of the RNA. Furthermore, we have used a variety of single-stranded probes to measure strand-specific transcription and to identify possible transcription blocks in the gene similar to the one found in *c-myc* (3). Genomic DNA fragments of the H-2Kb gene (Fig. 1A) were cloned into M13mp18 and M13mp19 bacteriophage vectors by standard procedures (20). The Kb 5' probe consisted of a 1.4-kilobase *HincII-PstI* fragment (–99 to +1280). The Kb 3' probe consisted of a 0.8-kilobase *PstI-PvuII* fragment extending from intron 4 up to 4 nucleotides after the TGA stop codon. A 0.9-kilobase 5' cDNA fragment of H-2Kd is not depicted in Fig. 1A. The Kb and Kd probes cross-hybridized with all class I transcripts. The single-stranded DNA clones were immobilized on nylon filters and hybridized with nuclear run-on RNA from Ad12 and Ad5 E1-transformed cells as described elsewhere (22). Filters were washed in 0.2× SSC–1% sodium dodecyl sulfate (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 50°C. Figure 1B shows that all class I DNA template (+) probes (i.e., Kb 5', Kb 3', and the Kd cDNA) yielded much stronger hybridization signals with RNA from Ad5 E1-transformed baby mouse kidney (BMK) cells than with RNA from Ad12 E1-transformed BMK cells. The nontemplate (–) probes did not show hybridization signals above background levels (M13 wild type), indicating that class I genes are transcribed in a strand-specific manner. Hybridization to clones carrying the rat glyceraldehyde 3-phosphate dehydrogenase cDNA showed that labeling efficiencies of the preparations of nuclei were comparable. Similar results were obtained by using the Kb 5', Kb 3', and cDNA fragments as double-stranded DNA probes (data not shown). Experiments using baby rat kidney (BRK) cells transformed by Ad5 or Ad12 E1 yielded similar differences (data not shown). Thus, the results indicate that the rate of transcription of MHC class I genes in Ad12 E1-transformed cells is repressed compared with that in Ad5 E1-transformed cells. These results are in contrast to our initial nuclear run-on data (29),

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FIG. 1. Nuclear transcription of MHC class I genes in isolated nuclei from Ad5 E1- and Ad12 E1-transformed BMK cells. (A) Structural organization of the H-2Kb gene. 1 to 8, Exons; 3' UT, 3' untranslated region. The position of the cap site (▶) is indicated. Probes represent genomic fragments corresponding to the 5' and 3' parts of the H-2Kb gene. (B) Hybridization of equivalent amounts (in counts per minute) of labeled run-on RNA from Ad5 E1- or Ad12 E1-transformed BALB/c (haplotype d) BMK cells to filters containing 1 µg of single-stranded DNA probes. Hybridization to the DNA template (coding) strand (+) or to the nontemplate strand (-) is indicated. M13, Single-stranded M13mp19 DNA; GAPDH, rat glyceraldehyde 3-phosphate dehydrogenase cDNA.

indicating that, at least in our hands, this type of experiment is sensitive to artifacts, particularly for genes directed by weak promoters.

Transgenic mice. In order to obtain additional support for our run-on data, we used transgenic mice carrying the H-2K-human growth hormone (hGH) fusion gene (21). The fusion gene contained the hGH-coding sequence placed under the control of an H-2K promoter. Two independent transgenic founder mice (mice 15 and 40; haplotype b) were crossed with C57BL/6 mice. The offspring of both crosses were screened for the presence of the hGH gene (data not shown), and from the hGH gene-positive animals primary BMK cells were prepared which were subsequently transformed by Ad5 E1 or Ad12 E1. To examine the level of expression of endogenous class I genes and of the H-2K-hGH fusion gene, total cytoplasmic RNA was extracted as described previously (6). RNA concentrations were measured spectrophotometrically and confirmed by agarose gel electrophoresis. The RNA was hybridized to the appropriate probes at 56°C and subjected to nuclease S1 digestion (13). The probes used to identify and discriminate the mRNAs of the endogenous MHC class I genes and of the fusion gene are depicted in Fig. 2B. The results of an experiment in

FIG. 2. Quantitative nuclease S1 analysis of the H-2Kb and H-2K-hGH expression in Ad5 E1- and Ad12 E1-transformed transgenic BMK cells. Cells from transgenic mice 2, 6, 8 (all derived from founder mouse 15) and 16 (derived from founder mouse 40) were used for transformation by Ad5 E1; mice 2 and 8 were used for transformation by Ad12 E1. (A) Uniformly labeled H-2Kb and hGH probes were hybridized to 20 µg of total RNA isolated from the Ad5 E1-transformed transgenic cell line tgX6.2, tgX2.2, tgX8.1, tgX6.1, or tgX16.2 (lanes 1 and 5 to 8, respectively); the Ad12 E1-transformed transgenic cell line tgR2.7, tgR8.6, or tgR8.7 (lanes 2 to 4, respectively); the Ad5 E1-transformed C57BL/6 BMK cell line (lane 9); or an Ad5 E1-transformed BALB/c BMK cell line (haplotype d) (lane 10). S, RNA isolated from the spleen of a transgenic mouse; M, marker fragments; I, undigested probes. (B) Schematic diagram of the H-2Kb and hGH probes and the expected protected segments.

which both probes were used simultaneously are shown in Fig. 2A. The three Ad12-transformed transgenic cell lines (lanes 2 to 4) all showed reduced amounts of endogenous class I gene transcripts compared with the four Ad5-transformed cell lines (lanes 1 and 5 to 8). Similarly, no detectable amount of transcripts of the H-2K-hGH fusion gene were found in the Ad12-transformed cell lines. In contrast, these

transcripts were readily detectable in the Ad5-transformed cell lines. Hybridization of RNA extracted from untransformed spleen cells of one transgenic mouse to both probes revealed the same protected Kb and hGH fragments (lane S). In Ad5 E1-transformed C57BL/6 BMK cells (haplotype b) which were not derived from transgenic mice the hGH band was absent (lane 9), while in Ad5 E1-transformed BALB/c BMK cells (haplotype d) both the hGH and the H-2Kb bands were absent (lane 10), indicating the specificity of the probes. This experiment showed that in Ad5 E1- and Ad12 E1-transformed cells, the H-2K-hGH fusion gene expression mimics that of the endogenous class I genes. These results in combination with the nuclear run-on data establish that the reduced expression of class I genes by Ad12 E1 is caused by repression of the activity of the H-2 promoter.

Conclusions. Several cellular genes have been found to be transcriptionally repressed by both Ad5 and Ad12 E1A. These genes include the JE gene (a platelet-derived growth factor-inducible gene), the gene coding for the protease stromelysin, and the muscle-specific α -actin genes (22, 27, 33). Furthermore, both viral and cellular enhancers, such as those of polyomavirus, simian virus 40, and the immunoglobulin enhancer, are also transcriptionally repressed by E1A in transient expression assays (7, 11, 30). Our results with the Ad12 E1-transformed BMK cells from transgenic mice carrying the H-2K promoter linked to a reporter gene confirmed the nuclear run-on data obtained by others and by ourselves (1, 9), which indicate that the repression of MHC class I genes occurs at the transcriptional level. Despite the resemblance, this repression differs in several aspects from that of the other genes. First, repression of MHC class I genes was observed only in Ad12 E1-transformed primary cells (i.e., when the cells were transformed as primary cultures), whereas repression of most of the other genes by Ad5 E1A and Ad12 E1A occurred in both primary cells and established cell lines. Second, there was a striking difference between the regions of E1A involved in these repressions. For most of the genes studied, it has been reported that repression is a function of conserved domains 1 and 2 (CR1 and CR2) shared by the 12S and 13S gene products (18, 19, 23). However, MHC class I gene repression is mediated by the 13S gene product only (6, 28), which, in addition to CR1 and CR2, has a third conserved domain (CR3) that is responsible for the *trans*-activating function of E1A. This may imply that the domain responsible for *trans*-activation and not the domain responsible for *trans*-repression by E1A is involved in the down regulation of MHC class I genes in transformed primary cells.

The mechanism of *trans*-activation by E1A is still unclear. Since E1A does not bind to DNA directly (8) and the promoters of genes that are *trans*-activated by E1A do not share specific sequence motifs other than motifs for common transcription factors (4, 14), the current model for E1A *trans*-activation is that E1A modifies the activity of transcription factors already present in untransformed cells (10). One of these factors could be a TATA-binding factor (17, 26, 34), but other transcription factors can be involved as well. When common transcription factors are involved in E1A functioning, the activity or the presence of such factors could also be important in the down regulation of MHC class I genes in naturally occurring tumors, e.g., neuroblastomas and melanomas (5, 31). Since the MHC class I promoter is characterized in some detail with respect to the binding of transcription factors (2, 12, 16, 25), future research will be directed toward the identification of the transcription factors which are altered by E1A and the elucidation of the mechanism by which E1A modifies the activity of these transcription factors.

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