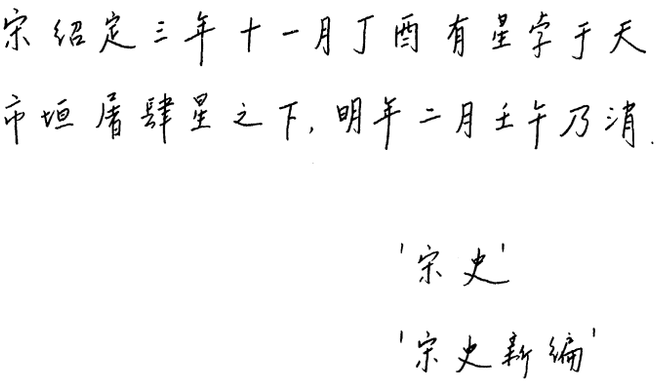

Fig. 3. Chinese record of appearance of the new star A.D. 1230. "(Sung) Reign Shao-Ding, Year 3, Month 11, Day Ding-you [15 December 1230], a po star appeared to the south of 109 Her at the Tieh-shih, until the Day Ren-wu of the second month of the following year [20 March 1231], it disappeared." [From *Sung-Shi* and *Sung-Shi-Xin-Bian*]

sion 19 hours 23 minutes and declination 19° 33′, ±1°. It is in the region where the new star of A.D. 1230 occurred. The ancient record and translation are shown in Fig. 3.

(Sung) Reign Shao-Ding, Year 3, Month 11, Day Ding-you [15 December 1230], a po star appeared to the south of 109 Her at the Tieh-shih, until the Day Ren-wu of the second month of the following year [20 March 1231], it disappeared.—From *Sung-Shi* and *Sung-Shi-Xin-Bian*

The ancient record described the new star of A.D. 1230 as a "po star," that is, a new star with visual magnitude of 3 to 4, and the duration of its appearance as 100 days. It might be considered as a supernova that occurred at a far distance, suffering strong absorption. The visible duration of longer than 6 months suggested by Clark and Stephenson (*12*) as a necessary condition for supernovae is certainly applicable for nearby supernovae. We expect distant supernovae to be fainter and of shorter observed duration. Of course, the chance for confusion with ordinary novae is greater for distant supernovae.

In view of the observation that the young compact remnants of supernova explosions such as the Crab and Vela pulsars emit gamma-ray radiation, it is reasonable to assume that some other young compact remnants of supernova explosions may also emit gamma-ray radiation. From the coincidence of visual positions between gamma-ray sources and ancient guest stars, identifications for 2CG 353+16 and 2CG 054+01 are suggested. More information about their distances, ages, or time signatures are needed to further strengthen these identifications.

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Post-Transcriptional Control of Class I MHC mRNA Expression in Adenovirus 12–Transformed Cells

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Expression of the class I transplantation antigens of the major histocompatibility complex (MHC) is suppressed in cells transformed by the oncogenic human adenovirus 12 (Ad12). This suppression of class I antigen expression, which contributes to the tumorigenic phenotype of the transformed cells, has also been observed in some naturally occurring cancers. In the present study, the rate of transcription initiation of class I genes was measured by a nuclear run-on assay in Ad5- and Ad12-transformed cells of three different types. The rate of transcription was the same in all three. The stability of the class I messenger RNA was also examined and found to be the same in all three cell types. The results indicate that in Ad12-transformed cells the suppression is caused by an inhibition of the post-transcriptional processing of class I MHC messenger RNA in the nucleus.

VIRTUALLY ALL CELLS OF HIGHER eukaryotes express class I transplantation antigens of the major histocompatibility complex (MHC) on their surfaces. These proteins play a crucial role in MHC-restricted cytolysis, a process in which cells expressing foreign antigens, for example, as a result of viral infection, are recognized and destroyed by cytotoxic T-lymphocytes. Previous studies in our laboratory showed that cells transformed by the oncogenic human adenovirus 12 (Ad12), as opposed to cells transformed by the non-oncogenic Ad5, express greatly reduced amounts of class I MHC antigens on their plasma membranes (*1, 2*). This phenomenon has also been observed in a number of naturally occurring cancers (*3–5*). Since the tumorigenicity of Ad12-transformed cells is lost if class I expression is restored (*6*), reduced expression of class I MHC antigens apparently contributes to the tumorigenic phenotype of the cells. We showed previously that the reduction of class I proteins on Ad12-transformed cells is accompanied by a decrease in the level of cytoplasmic class I messenger RNA (mRNA) (*1, 2*). This is confirmed in Fig. 1A, which shows the steady-state levels of the mature cytoplasmic 1.6-kb class I mRNA in untransformed, Ad5-transformed and Ad12-transformed BALB/c baby mouse kidney (BMK) cells, as measured by Northern blotting.

To investigate whether the reduction of class I MHC mRNA concentration is caused by a suppression of RNA transcription, we measured the rate of transcription initiation of class I genes by the use of a nuclear run-on assay with nuclei from the same cells that were used for the experiment in Fig. 1A. The rate of transcription initiation of class I genes in vivo, reflected by the amount of labeled RNA hybridizing to a mouse H-2 class I complementary DNA (cDNA), was the same in the three cell types (Fig. 1B). Similar transcription initiation rates were also found for the γ-actin and β-tubulin genes, which were measured as controls. (The cytoplasmic concentrations of the

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mRNA for these genes are the same in Ad5- and Ad12-transformed cells.) Similar results were obtained with Ad5- and Ad12-transformed BMK cells from the C57/Bl mouse strain. Furthermore, when we carried out the same experiment using single-stranded H-2 probes, we found that only the sense strand was transcribed, and at similar rates, both in untransformed and in adenovirus-transformed BALB/c and C57/Bl BMK cells. The reduced concentration of cytoplasmic class I mRNA in Ad12-transformed BMK cells was also found in Ad12-transformed human embryonic retinoblast cells (*2*). Therefore, we also measured transcription initiation rates of class I genes in the latter cells. As in the murine system, the transcription initiation rates of class I genes were similar in Ad5- and Ad12-transformed human cells (Fig. 1C). Since identically prepared nuclei allowed us to measure transcriptional differences for several other genes (*7, 8*), we conclude that the difference in cytoplasmic class I mRNA concentration, between Ad5- and Ad12-transformed cells of murine or human origin, is not caused by differences in the rate of transcription initiation of class I genes in these cells.

Recently, a number of investigators have described post-transcriptional control mechanisms in which cellular gene expression was regulated by modulation of cytoplasmic mRNA stability (for example, for p53 and c-*myc*) (*9*). This led us to investigate whether the difference in cytoplasmic class I mRNA concentration between untransformed and Ad5-transformed BMK cells on one hand and Ad12-transformed BMK cells on the other could be attributed to differences in the stability of class I mRNA. Cytoplasmic RNA was extracted after incubation of the cell cultures for various times with actinomycin D, and subjected to Northern blot analysis to determine the relative amounts of class I mRNA. Since actinomycin D blocks RNA synthesis, the decrease in mRNA concentrations caused by its presence should be a measure of RNA stability. Figure 2A shows that, within 10 hours of treatment with actinomycin D, the stability of class I mRNA is the same in all three cell types. (Note that we applied approximately three times as much RNA from Ad12-transformed cells to the gel as from untransformed and Ad5-transformed cells.) The half-life of the Ad12E1A and E1B mRNAs under these conditions was approximately 1 hour, as shown in Fig. 2B (the same result was obtained for the Ad5E1 mRNAs). E1A proteins are relatively unstable molecules (Ad5E1A proteins have a half-life of approximately 30 minutes in infected cells and 120 minutes in Ad5-transformed 293 cells) (*10*). Hence, actinomycin D treatment will affect the concentration of E1A proteins and possibly other unstable proteins, which are likewise involved in the down-regulation of class I expression, if they are also translated from short-lived mRNAs. Therefore, incubation with actinomycin D for periods longer than a few hours is probably ineffective if one wishes to estimate class I mRNA stability in adenovirus-transformed cells. Nevertheless, the results suggest that the difference in cytoplasmic class I mRNA concentration between Ad12-transformed cells and untransformed or Ad5-transformed cells is not caused by a decreased cytoplasmic stability of the class I mRNA in Ad12-transformed cells.

The data from the nuclear run-on and actinomycin D experiments suggest that a post-transcriptional process in the nucleus of Ad12-transformed cells has been altered compared to untransformed and Ad5-transformed cells. Although little is known about the control of gene expression at this level, it has been suggested, for instance, that dihydrofolate reductase gene expression in mouse cells is regulated by transcript stabilization in the nucleus (*11*). In an attempt to identify differences in the nuclear processing of class I transcripts between Ad5- and Ad12-transformed BMK cells, nuclear RNA from both cell types was isolated and subjected to Northern blot analysis with a homologous class I cDNA (*12*) probe. Figure 3A shows that the nuclear concentration of class I RNA is also drastically reduced in Ad12-transformed cells. Apart from the 1.6-kb mature class I mRNA, several RNA molecules with higher molecular weights, possibly representing splicing intermediates of class I mRNA, hybridized to the class I cDNA probe. The ratio of these pre-mRNA molecules in Ad5- and Ad12-transformed cells is comparable to that of the mature 1.6-kb nuclear class I mRNA (see arrows in Fig. 3, A and B). Hybridization of the same filter with a mouse γ-actin cDNA probe showed that the lanes contain comparable amounts of RNA (Fig. 3C). This was supported by the ethidium bromide staining of the RNA samples (Fig. 3D). Since the transcription initiation rate of class I genes in Ad5- and Ad12-transformed cells is equal (Fig. 1, B and C), the results suggest that the decreased levels of class I mRNA in the nucleus and cytoplasm of Ad12-transformed cells are caused by post-transcriptional degradation, for example, during processing of pre-mRNA to mature mRNA.

Our observation that class I MHC repression in Ad12-transformed cells does not occur at the level of transcription initiation is confirmed by Khoury and co-workers

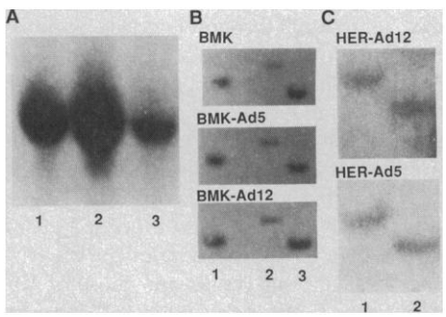


Fig. 1. (**A**) Cytoplasmic RNA (20 μg) from untransformed (lane 1), Ad5-transformed (lane 2), and Ad12-transformed (lane 3) BMK cells analyzed by Northern blotting (*1*) with a homologous class I probe (*26*). (**B** and **C**) In vitro run-on transcription in isolated nuclei [adapted from Friedman *et al.* (*27*)]. After the cell cultures were trypsinized, they were maintained at 4°C. Cells were washed with phosphate-buffered saline and homogenized (Dounce method, five to ten strokes, tight pestle) in 10 m*M* tris (*p*H 7.8), 5 m*M* $MgCl_2$, 150 m*M* KCl, and 5 m*M* dithiothreitol (1× TMKD) and 0.1% NP-40. After centrifugation of the homogenate (600*g*, 3 minutes), the nuclear pellet was washed in 1× TMKD, 10% glycerol, and 1 m*M* $MnCl_2$, and 10^7 nuclei were gently resuspended in 75 μl of reaction buffer (1× TMKD, 10% glycerol) and kept on ice. Reaction buffer (30 μl of 5× buffer) was added to 1.2 mCi (120 μl) of [α-^{32}P]uridine 5′-triphosphate (UTP) (Amersham) and mixed. Portions (25 μl) of this UTP solution (200 μCi) were added to the nuclei in suspension. Cold UTP was added to reach a final concentration of 4 m*M*, and the other nucleotide triphosphates and $MnCl_2$ were added to reach final concentrations of 250 μ*M* and 1 m*M*, respectively. The nuclei suspensions (four independent suspensions were prepared) were incubated under regular whirling (30°C, 30 minutes) and centrifuged (Eppendorf, 15 seconds, room temperature), and the pellet was suspended in 300 μl of deoxyribonuclease (DNase) buffer [20 m*M* Hepes (*p*H 7.5), 5 m*M* $MgCl_2$, and 1 m*M* $CaCl_2$]. RQ1 DNase (Promega Biotec) was added to reach a final concentration of 20 μg/ml and incubated at 37°C for 5 to 10 minutes. Extraction of RNA from the nuclei was done according to McKnight and Palmiter (*28*). Equivalent amounts (counts per minute) of labeled RNA from the various cell types were hybridized to GeneScreenPlus filters (New England Nuclear) containing different cDNA inserts, in a buffer containing 40% formamide, 20 m*M* 1, 4-piperazinediethanesulfonic acid (Pipes) (*p*H 7.5), 1% SDS, 0.5*M* NaCl, and *Escherichia coli* transfer RNA (200 μg/ml). Hybridization was for 48 to 72 hours at 42°C. Washing of the filters was in 2× standard saline citrate/0.1% up to 60°C, and included a ribonuclease A digestion (10 μg/ml) for 30 minutes at 37°C. (**B**) Run-on RNA from untransformed BMK cells and Ad5- and Ad12-transformed BMK cells hybridized against filters containing mouse γ-actin (lane 1) (*29*), chicken β-tubulin (lane 2) (*30*), and mouse class I (lane 3) (*26*) cDNA inserts. (**C**) Run-on RNA from Ad5- and Ad12-transformed human embryonic retinoblast (HER) cells hybridized against filters containing mouse γ-actin (lane 1) (*29*) and human HLA-B7 (lane 2) (*31*) cDNA inserts.

Fig. 2. Actinomycin D treatment of untransformed, Ad5- and Ad12-transformed BMK cells. Actinomycin D (5 μg/ml; Calbiochem) was added to the medium of the cell cultures for the indicated periods. (**A**) Cytoplasmic RNA of 2×10^6 BMK and Ad5 = BMK cells (20 μg), and 6×10^6 Ad12-BMK cells (60 μg) of each time point was subjected to Northern blotting analysis, with a homologous class I probe (*25*). (**B**) Northern analysis of cytoplasmic RNA from 2×10^6 Ad12-BMK cells (20 μg) with the Eco RI Ad12E1 fragment isolated from pAd12RIC (*32*) as probe.

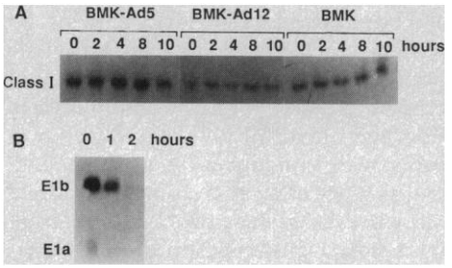
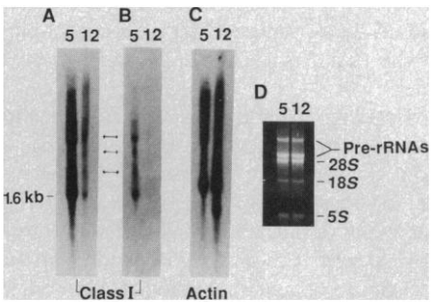


Fig. 3. Northern analysis of nuclear RNA extracted from Ad5- Ad12-transformed BMK cells. Nuclei from Ad5- and Ad12-transformed BMK cells were prepared as described in the legend to Fig. 1. After washing in 1× TMKD–10% glycerol, nuclei were mixed vigorously in 3*M* LiCl/6*M* urea. After precipitation overnight at 4°C, the RNA was pelleted by centrifugation (10,000 rpm in Sorvall HB-4 rotor at 4°C). The pellet was washed once with LiCl/urea, once with 70% ethanol, and dissolved in 10 m*M* tris-chloride (*p*H 7.8), 5 m*M* EDTA, and 1% SDS. Proteinase K (Boehringer) was added to achieve a final concentration of 100 μg/ml; digestion was for 1 hour at 37°C. The solution was extracted once with phenol:chloroform:isoamyl alcohol and once with chloroform:isoamyl alcohol, and the RNA was precipitated with two volumes of ethanol at −20°C. (**A**) Equivalent amounts (20 μg) of the nuclear RNA samples were subjected to Northern blot analysis by means of a homologous class I cDNA probe (*26*). (**B**) Shorter exposure of the filter shown in (A). (**C**) The same filter shown in (A) and (B) was stripped in H_2O) (30 minutes, 80°C), and hybridized with a nick-translated mouse γ-actin cDNA fragment (*29*). (**D**) Equivalent amounts of the nuclear RNA samples, subjected to electrophoresis in a sterile ethidium bromide–containing agarose–tris borate EDTA gel; rRNAs, ribosomal RNAs.



(*13*). Furthermore, it agrees with results of Tanaka *et al.* (*6*) who have shown that the low expression of transfected class I genes in stable transfectants, which had been obtained after introduction of class I genes into Ad12-transformed mouse cells, is not dependent on the nature of the promoter region in front of the class I gene.

The available information on the function or functions of region E1A, based mainly on transient expression assays and experiments with lytically infected cells, indicates that E1A products can modulate gene expression by either activating (*14–17*) or suppressing (*18, 19*) transcription. Although the actual mechanism by which E1A stimulates transcription is still unknown, Yoshinaga *et al.* (*20*) have recently shown that Ad5E1A stimulates RNA polymerase III transcription from exogenous DNA templates in extracts of infected cells by increasing the concentration of transcription factor IIIC, which is normally present in limiting amounts. A similar mechanism may also explain the E1A-induced activation of transfected genes transcribed by RNA polymerase II (*17*). However, RNA levels of endogenous cellular genes that are transcribed by RNA polymerase III, and most endogenous genes transcribed by RNA polymerase II, are not activated by E1A (*20–23*). This may be explained by the fact that DNA introduced into a cell through infection or transfection is not organized in an ordered chromatin structure, at least not within the first 2 days (*17*), and that this "naked" DNA is more readily accessible to soluble transcription factors than endogenous cellular genes. This "nonspecific" stimulatory effect of the E1A region on transiently expressed genes may also be the explanation for the discrepancy between our results and those of Kimura *et al.* (*24*), who found, in transient expression experiments, that chloramphenicol acetyltransferase gene constructs driven by the promoter region of an H-2 class I gene were approximately tenfold less active in Ad12-transformed cells than in Ad5-transformed cells. Another result in apparent conflict with ours was reported by Rosenthal *et al.* (*25*), who showed that infection of mouse embryo cells with Ad12 virions leads to E1A-dependent stimulation of class I gene expression, which could be attributed in part to transcriptional activation. Apparently, Ad12 modulates class I gene expression differently in lytically infected and stably transformed cells.

In the present study, we have shown that Ad12E1A does not suppress class I MHC gene expression in Ad12-transformed cells by reducing the rate of transcription initiation, but by influencing a post-transcriptional process within the nucleus. Stages in the maturation of class I mRNA that could be sensitive to down-regulation are splicing and polyadenylation. Interference with one of these processes could result in a decrease in the stability of the class I (pre)mRNA. A block in the transport of class I mRNA seems unlikely, since no accumulation of either pre-mRNA or mature mRNA is observed in nuclei of Ad12-transformed cells (Fig. 3A).

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