Increased MHC H-2K gene transcription in cultured mouse embryo cells after adenovirus infection

Aron Rosenthal*, Stephanie Wright*, Kristina Quade*, Phillip Gallimore§, Howard Cedar† & Frank Grosveld*

* Laboratory of Gene Structure and Expression, and † Division of Virology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK
‡ Department of Molecular Biology, Hadassah Medical School, The Hebrew University, Jerusalem, Israel
§ Department of Cancer Studies, CRC Laboratory, University of Birmingham, Birmingham B15 2TJ, UK

The class I major histocompatibility complex (MHC) antigens are highly polymorphic cell-surface proteins whose expression is essential for the cellular immune response against virus-infected, abnormal and foreign cells. Transformation of primary rat cell cultures by the oncogenic adenovirus 12 (Ad12) results in suppression of the transplantation antigens, thus enabling the transformed cells to escape the immune response and efficiently form tumours in vivo. In contrast, transformation of the same cells with the non-oncogenic adenovirus 5 (Ad5) does not suppress the transplantation antigens and, consequently, they elicit an effective (MHC-restricted) immune response. Here, however, we show that infection of mouse embryo cells with both viruses initially increases the level of transcripts from the H-2K gene. The adenovirus E1a (12S RNA) and E1B genes are required for activation of the H-2K gene and measurement of the relative rate of transcription indicates that the increase in the level of H-2K messenger RNA following infection is at least in part due to a gene-specific transcriptional activation. The newly transcribed H-2K mRNA is then properly transported to the cytoplasm.

Cell cultures from 16-day-old C57BL/10 mouse embryos were infected after second or third passage with wild type or mutants of Ad5. RNA was extracted 50 h after infection and the level of transcripts from the H-2K gene measured by S1 nuclelease protection analysis using a 600-nucleotide AUC1 probe which spans the exon III-intron III boundary of the H-2K gene. As indicated by the relative intensities of the 230-nucleotide S1 nuclelease-protected fragment (Fig. 1a), infection with Ad5 resulted in a 10-15-fold increase in the level of H-2K mRNA (lane 2) compared with mock-infected cells (lane 1). In contrast, infection with either the Ad5 d312 E1a deletion mutant or the Ad5 d313 E1B deletion mutant did not affect an increase in the level of H-2K mRNA (Fig. 1a, lanes 3, 4). To determine whether Ad5 infection caused a nonspecific change in the rate of gene transcription and/or RNA stability, we also measured the level of histone H4 gene transcripts in the above experiments. The level of histone H4 RNA increased only 2-3-fold after Ad5 infection under conditions in which the level of H-2K gene transcripts increased 15-fold (Fig. 1b).

Measurement of the relative rate of transcription of H-2K in nuclei isolated from mock-infected and Ad5-infected cells indicated that the increased level of H-2K mRNA following Ad5 infection was at least in part due to transcriptional activation. The activation appeared to be specific for H-2K as the rate of transcription of the type IV collagen gene did not increase on Ad5 infection (Fig. 1d).

Infection of mouse embryo cultures with Ad12 also resulted in a 10-15-fold increase in the level of total cellular H-2K mRNA (Fig. 2a, lane 1) compared with mock-infected cells (lane 9). Infection with a non-tumorigenic Ad12 E1a mutant (in 751), which lacks functional protein products from both the 12S and 13S mRNAs because of a stop codon in the first exon, did not activate the H-2K gene (Fig. 2a, lane 8). Similarly, infection with a non-tumorigenic Ad12 mutant (hr1121) which does not produce the 52K (relative molecular mass 52,000) E1B protein did not induce H-2K gene expression (Fig. 2a, lane 5). Infection with a weakly tumorigenic Ad12 E1a mutant...
Fig. 2  a, b, S1 nuclease analysis of H-2Kb (a) and histone H4 (b) mRNA from mock-infected (lane 9) C57BL/10 cells and from cells infected with 10-20 PFU per cell of Ad12 Ela mutant hr751 (lane 7), Ad12 Ela mutant in600 (lane 6), Ad12 Elb mutant hr1121 (lane 5), in751 plus hr1121 (lane 3), hr751 plus in600 (lane 4), in600 plus hr1121 (lane 2) and Ad12 wild type (lane 1). S1 nuclease analysis was done as described for Fig. 1a. b, c, S1 nuclease analysis of H-2Kb mRNA from mock-infected C57BL/10 cells (lane 1), control tRNA (lane 2) and from cells infected with Ad12 (lane 3), in751 (lane 4), hr771 (lane 5), in600 (lane 6) and Ad12 hr1121 (lane 7) at a titre of 50 PFU per cell. S1 nuclease analysis was done as described for Fig. 1a, d. Dot hybridization of labelled nuclear transcripts from mock-infected (-) and Ad12-infected (+) C57BL/10 mouse embryo cells to H-2Kb and type IV collagen gene fragments. Methods as for Fig. 1c.

Fig. 3  a, S1 nuclease analysis of H-2Kb gene transcripts from mock-infected C57BL/10 mouse embryonic cells (lane 4), control tRNA (lane 5) and from cells infected with polyoma virus (lane 1) or SV40 (lane 2) at a multiplicity of infection of 10-50 PFU per cell. Lane 3 contains control RNA isolated from C57BL/10 cells treated with 1,000 U ml-1 mouse γ-interferon. b, S1 nuclease analysis of cytoplasmic (cyt) and nuclear (nuc) H-2Kb mRNA from mock-infected (lane 2), Ad5-infected (lane 3, 10 PFU per cell) and Ad12-infected (lane 4, 10 PFU per cell) C57BL/10 mouse embryonic cells. Lane 1 contains a tRNA control. S1 nuclease analysis was carried out as described for Fig. 1. All lanes contain an equal amount of RNA (20 μg).

(hr771) which produces some Ela protein products only after a delay in permissive cells10, or with an Ad12 Ela mutant (in600) which contains the normal Ela 12S RNA and a truncated 13S RNA10 resulted in, respectively, no increase (Fig. 2a, lane 7) or only a marginal increase (lane 6) in the level of H-2K gene transcripts at the multiplicity of infection used (10 plaque-forming units (PFU) per cell). Co-infection of Ela mutants hr751 and in600 with each other or with the hr1121 Elb mutant resulted in an increase in the level of H-2K mRNA (Fig. 2a, lanes 4, 3 and 7 respectively): this effect could be caused by either complementation or an increased number of virus particles (10 PFU per cell of each; see below). The levels of histone H4 transcripts in these infections were only 1.5-2 times higher than in mock-infected cells (Fig. 2b).

Failure of adenovirus Ela mutants to activate the H-2Kb gene may result from the lack of production of other adenovirus early proteins which are normally activated by the Ela gene products11,12. We therefore repeated the Ad12 mutant infections at a higher multiplicity of infection (50 PFU per cell) to allow production of other adenovirus early proteins in the absence of either complementation or an increased number of virus particles (10 PFU per cell of each; see below). The levels of histone H4 transcripts in these infections were only 1.5-2 times higher than in mock-infected cells (Fig. 2b).
the E1a proteins (ref. 13 and R. Grand and P. Gallimore, personal communication). As shown in Fig. 2c, infection with a high titre of E1a mutants (in600 and hr771) resulted in activation of the H-2Kβ gene equal to that effected by wild-type virus. This result indicates that it is not the protein products of the E1a 13S transcript alone that activate the H-2Kβ gene. The E1b 52K protein and the products of the E1a 12S RNA, or 13S exon 1 RNA, are directly or indirectly necessary for H-2Kβ gene activation since high-titre infection with either the E1b 52K mutant (hr1121) or the E1a mutant which lacked protein products of both 12S and 13S RNAs (hr751) failed to significantly (less than twofold) increase the level of H-2Kβ gene transcripts (Fig. 2c). Measurement of the relative rate of transcription of the H-2Kβ gene in mock-infected and Ad12-infected cells indicated that the increase in the level of H-2K mRNA following viral infection was at least in part due to transcriptional activation (Fig. 2d).

This result may reflect the trans-acting enhancing properties of the E1a protein products, which can activate viral15,16,18 newly introduced15-18, integrated19 and endogenous genes20 either directly or through inactivation of cellular repressor proteins11,12,19. Alternatively, the gene activation may be mediated by a general anti-viral state induced by cellular proteins in response to either viral replication or viral proteins21,22; this seems unlikely, as Ad12 virus infections at 10 PFU per cell did not result in detectable levels of interferon (<4 U ml-1) in the cell culture (data not shown).

The results presented here indicate that infection of mouse embryonic cells with either Ad5 or Ad12 results in transcriptional activation of the MHC H-2K gene. Viral protein products from the 12S E1a mRNA or the first exon of the E1a gene, which is common to both 13S and 125S mRNAs, and the 52K E1B protein are necessary for this activation (Fig. 2). As yet, we do not know whether viral protein products from both the E1a and E1b regions are mediating the activation directly or via interaction with other (early) viral or cellular proteins. For example, the Ad5 E1b 58K protein binds the same p53 cellular protein as simian virus 40 (SV40) large-T antigen24. To determine whether other DNA tumour viruses such as SV40 mediate induction of the class I MHC genes using similar mechanisms25 and whether induction is related to permissiveness of the virus/cell system, we infected mouse embryonic cells with either SV40 (non-permissive, as is adenoavirus) or polyoma virus (permissive). Infection with either of these viruses resulted in activation of the H-2K gene but at a lower level than that resulting from the adenoavirus infections, which itself was lower than that induced by interferon in a control experiment (Fig. 3a).

Our results are in contrast to those of Schrier et al.15 who reported a decrease in the level of cytoplasmic class I mRNA and suppression of MHC antigen expression in Ad2-, but not in Ad5-infected cells. As Schrier et al. measured cytoplasmic mRNA, the difference could be due to a block in the transport of the newly synthesized mRNA from the nucleus to the cytoplasm. To test this possibility, the cells were infected with Ad5 and Ad12 (10 PFU per cell) and both the nuclear and cytoplasmic mRNA were quantitated. The results (Fig. 3b) show that the H-2K mRNA in the cytoplasm is increased between 10 and 15-fold in both cases, while the mRNA level in the nucleus is much lower and only visible after a longer exposure, which excludes the possibility of a block in mRNA transport.

However, preliminary experiments using radioimmune labeling of the Ad5- and Ad12-infected cells indicate that the amount of H-2Kβ antigen on the cell surface increases only marginally, if at all, despite the large increase in cytoplasmic H-2Kβ mRNA (data not shown). Thus, the results suggest that infection first induces a stimulation of MHC transcription, which normally would enable a better cellular immune response against infected cells. In the case of viruses, for example AdS and Ad12, this may not result in an increased expression of MHC antigens on the cell surface because of other viral factors19. In the case of oncogenic viruses such as Ad12, this may be followed by a suppression of MHC class I gene transcripts to allow escape from immune surveillance. Whether this is caused by an immunity from natural killer cells26-28 alone and/or from cytotoxic T cells29 as part of an MHC-restricted response30, is unclear.

This work was supported by the BMRG (UK) and the Cancer Research Campaign. A.R. was supported by the Michael and Anna Wix Trust through the Friends of the Hebrew University; S.W. was self-supported. We thank Barry Els for gifts of SV40 and polyoma virus, D. Goeddel for mouse γ-interferon, J. Williams for the adenoavirus mutants hr771 and hr1221, B. Hogan for the collagen IV plasmid, M. Busslinger for the histone H4 plasmid, F. Balkwill for the interferon assays and K. Hui for the antibody binding assays.

Received 7 March; accepted 1 April 1985.

Irreversible swelling of secretory granules during exocytosis caused by calcium

Joshua Zimmerberg* & Michael Whitaker†

* Physical Science Laboratory, Division of Computer Science and Technology and Laboratory of Chemical Biology, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20295, USA
† Department of Physiology, University College London, Gower Street, London WC1E 6BT, UK

The fusion of the limiting membrane of a secretory granule with the plasmalemma during exocytosis is equivalent to the fusion and release of contents that occurs when phospholipid vesicles fuse with planar bilayers31,32. Experiments with bilayers demonstrate that phospholipid vesicles must swell if they are to fuse3. Also, inhibition of exocytosis in solutions of high osmolality occurs in several types of secretory cell1-8. We report here experiments on the cortical granule exocytosis of sea-urchin eggs. Exocytosis is prevented when the osmolality of the medium surrounding the eggs is raised from 1 to 2 osmol kg-1. High osmolality also prevents calcium-dependent exocytosis in vitro. Prior treatment with calcium at high osmolality triggers fusion when normal osmolality

1 To whom correspondence should be addressed.