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

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The class I major histocompatibility complex (MHC) antigens are highly polymorphic[1] cell-surface proteins[2] whose expression is essential for the cellular immune response against virus-infected, abnormal and foreign cells[3,4]. Transformation of primary rat cell cultures by the oncogenic adenovirus 12 (Ad12) results in suppression of the transplantation antigens[5], thus enabling the transformed cells to escape the immune response and efficiently form tumours *in vivo*[6]. In contrast, transformation of the same cells with the non-oncogenic adenovirus 5 (Ad5) does not suppress the transplantation antigens[5] and, consequently, they elicit an effective (MHC-restricted) immune response[6]. Here, however, we show that infection of mouse embryo cells with both viruses initially increases the level of transcripts from the *H–2K*^{b} transplantation antigen gene. Both 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*^{b} 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*^{b} gene measured by S_1 nuclease protection analysis using a 600-nucleotide *Ava*II probe which spans the exon III-intron III boundary of the *H-2K*^{b} gene[7]. As indicated by the relative intensities of the 230-nucleotide S_1 nuclease-protected fragment (Fig. 1*a*), infection with Ad5 resulted in a 10–15-fold increase in the level of *H-2K*^{b} mRNA (lane 2) compared with mock-infected cells (lane 1). In contrast, infection with either the Ad5 *dl*312 E1a deletion mutant or the Ad5 *dl*313 E1b deletion mutant[8,9] did not effect an increase in the level of *H-2K* mRNA (Fig. 1*a*, 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*^{b} gene transcripts increased 15-fold (Fig. 1*b*).

Measurement of the relative rate of transcription of *H-2K*^{b} 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. 1*c*).

Infection of mouse embryo cultures with Ad12 also resulted in a 10–15-fold increase in the level of total cellular *H-2K*^{b} mRNA (Fig. 2*a*, 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*^{b} gene (Fig. 2*a*, lane 8). Similarly, infection with a non-tumorigenic Ad12 mutant (*hr*1121) which does not produce the 52K (relative molecular mass 52,000) E1b protein[10] did not induce *H-2K* gene expression (Fig. 2*a*, lane 5). Infection with a weakly tumorigenic Ad12 E1a mutant

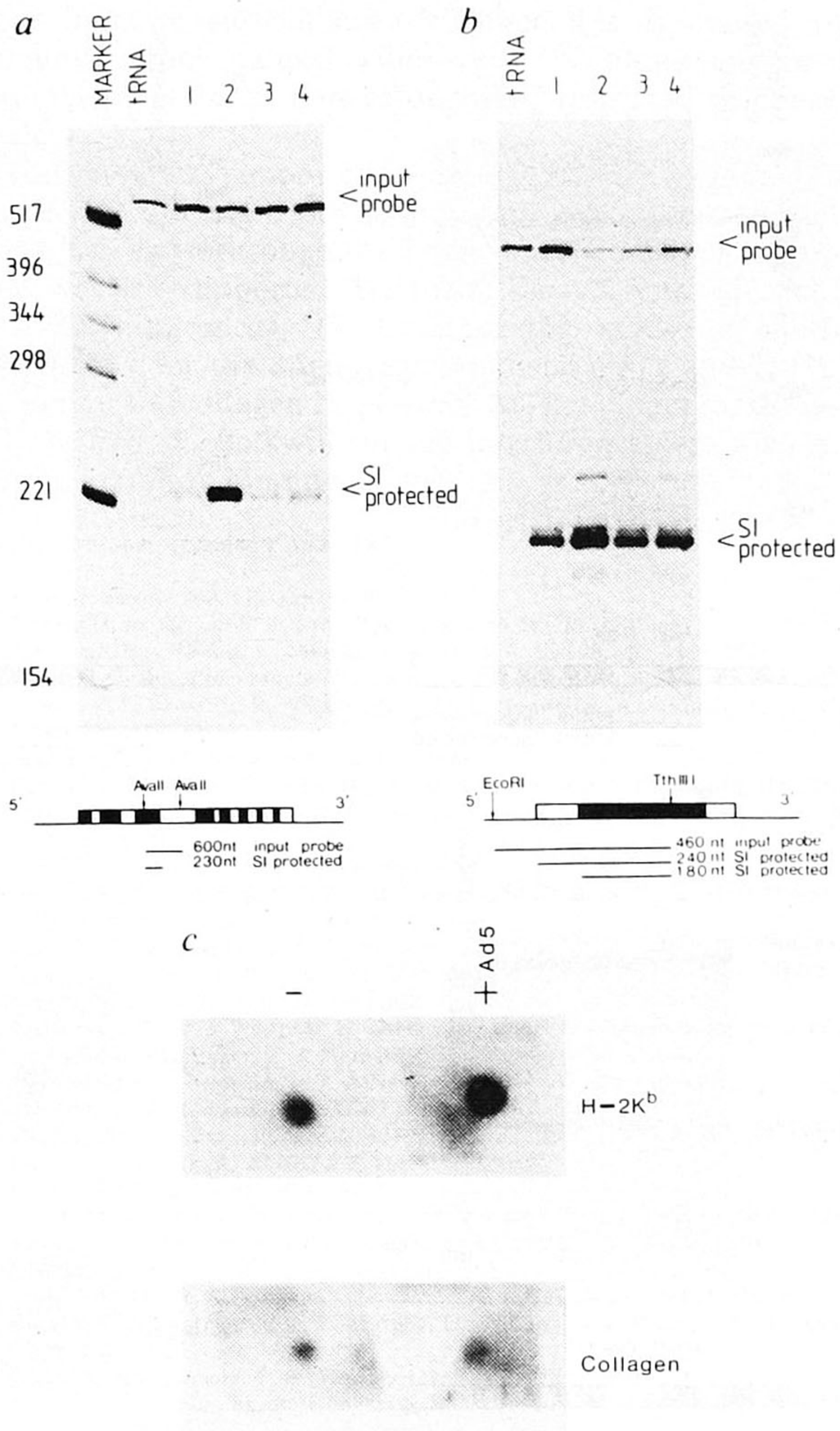

Fig. 1 *a*, *b*, S_1 nuclease analysis of *H-2K*^{b} (*a*) and histone H4 (*b*) mRNA from C57BL/10 mock-infected (lane 1), Ad5-infected (lane 2), *dl*312-infected (lane 3) and *dl*313-infected (lane 4) mouse embryo cells. Markers are ^{32}P-labelled pBr 322XHinf. *c*, Dot hybridization of labelled nuclear transcripts from mock-infected (–) and Ad5-infected (+) C57BL/10 mouse embryo cells to *H-2K*^{b} and type IV collagen gene fragment.

Methods. *a*, A 600-nucleotide probe spanning the exon III/intron III boundary of the *H-2K*^{b} gene was end-labelled with reverse transcriptase and hybridized with 15 μg total RNA at 60 °C for 12 h in 10 μl of 80% formamide, 40 mM PIPES *p*H 6.4, 1 mM EDTA, 400 mM NaCl. The mixture was digested with 3,000 units of S_1 nuclease (Boehringer) in 300 μl of 300 mM Na-acetate *p*H 4.8, 200 mM NaCl, 2 mM $ZnSO_4$ at 20 °C for 1 h, followed by 40 °C for a further hour. S_1 nuclease-protected DNA was ethanol-precipitated, denatured and electrophoresed on a 7 M urea/7% acrylamide gel[30,31]. *b*, A 460-nucleotide probe spanning the 5′ end of the histone H4 gene was end-labelled with T4 polynucleotide kinase and hybridized with 15 μg total RNA at 52 °C as described for *a*. Samples were digested with S_1 nuclease for 2 h at 20 °C, then treated as for *a*. *c*, Nuclei were isolated from mock-infected and Ad5-infected cells. RNA synthesized in 5×10^6 isolated nuclei was labelled for 20 min with 200 μCi [α-^{32}P]UTP (3,000 Ci mmol^{-1}) in a 300 μl reaction volume as described in ref. 32. Plasmids containing the *H-2K*^{b} gene and the type IV collagen gene were linearized by digestion with *Hin*fI and *Eco*RI respectively. DNA (15 μg) was bound to a 0.45-μm nitrocellulose filter[33] and hybridized to labelled RNA from 2×10^6 isolated nuclei in a 400-μl volume[32]. After 46 h, hybridized filters were washed at 54 °C in 0.1 × S.S.C., 0.1% S.D.S. for 45 min, air-dried and exposed for 2 h.

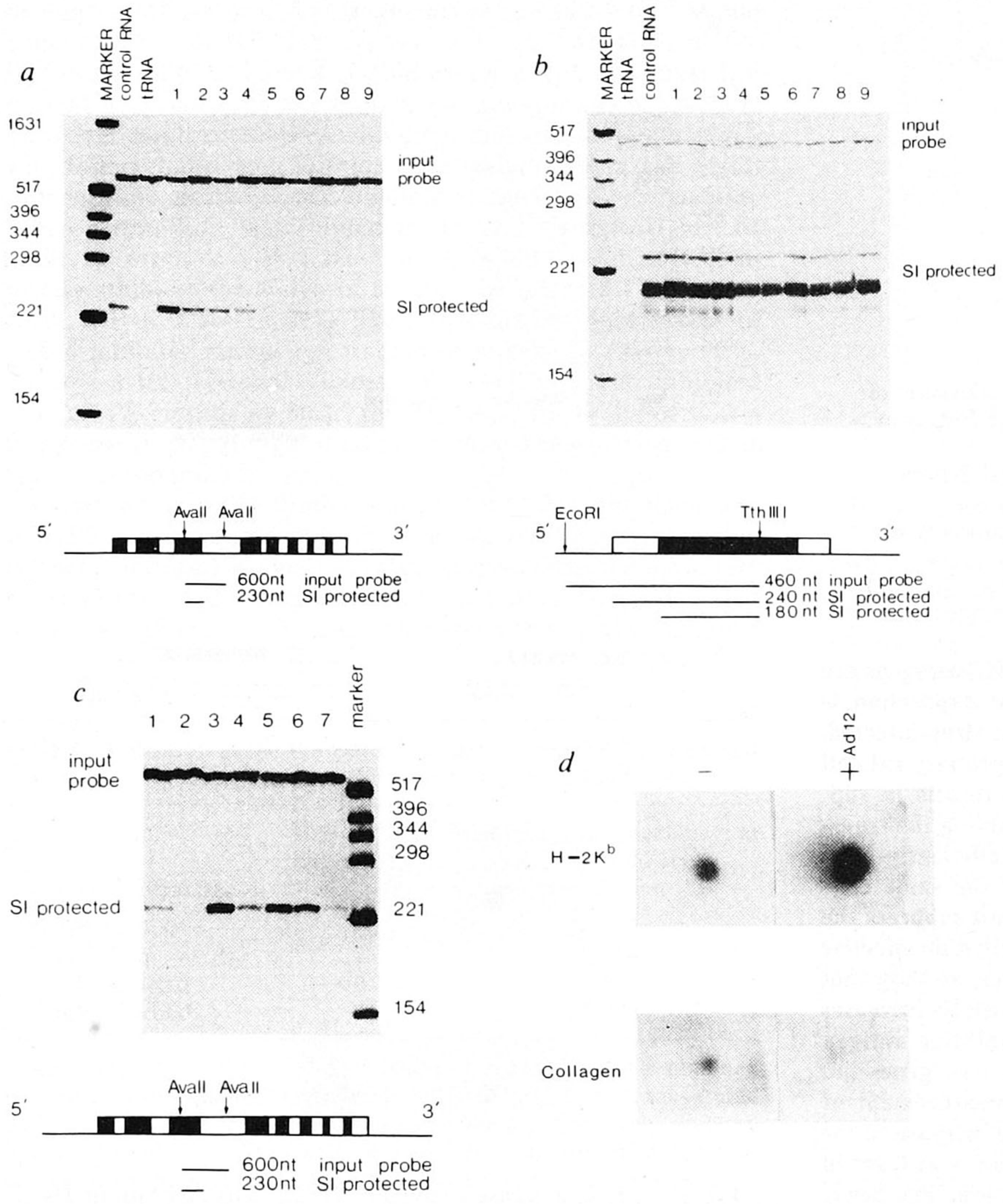

Fig. 2 *a*, *b*, S_1 nuclease analysis of *H-2K*^{b} (*a*) and histone H4 (*b*) mRNA from mock-infected (lane 9) C57BL/10 cells and from cells infected with 10–20 PFU per cell of Ad12 E1a mutant *hr*751 (lane 8), Ad12 E1a mutant *hr*771 (lane 7), Ad12 E1a mutant *in*600 (lane 6), Ad12 E1b mutant *hr*1121 (lane 5), *in*751 plus *hr*1121 (lane 3), *hr*751 plus *in*600 (lane 4), *in*600 plus *hr*1121 (lane 2) and Ad12 wild type (lane 1). S_1 nuclease analysis was done as described for Fig. 1*a*, *b*. *c*, S_1 nuclease analysis of *H-2K*^{b} mRNA from mock-infected C57BL/10 cells (lane 1), control tRNA (lane 2) and from cells infected with Ad12 (lane 3), *in*751 (lane 4), *hr*771 (lane 5), *in*600 (lane 6) and Ad12 *hr*1121 (lane 7) at a titre of 50 PFU per cell. S_1 nuclease analysis was done as described for Fig. 1*a*. *d*, Dot hybridization of labelled nuclear transcripts from mock-infected (–) and Ad12-infected (+) C57BL/10 mouse embryo cells to *H-2K*^{b} and type IV collagen gene fragments. Methods as for Fig. 1*c*.

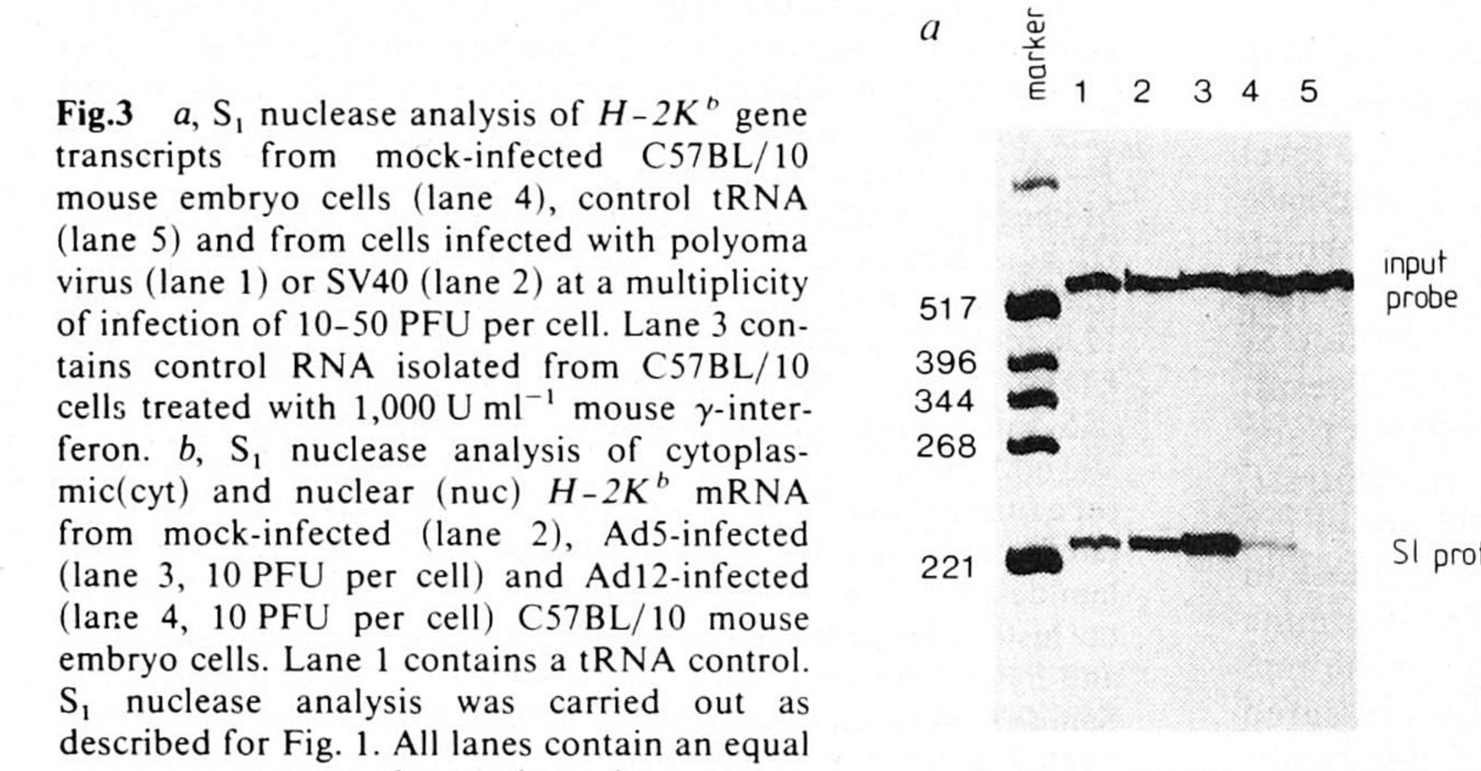

Fig.3 *a*, S_1 nuclease analysis of *H-2K*^{b} gene transcripts from mock-infected C57BL/10 mouse embryo cells (lane 4), control tRNA (lane 5) and from cells infected with polyoma virus (lane 1) or SV40 (lane 2) at a multiplicity of infection of 10–50 PFU per cell. Lane 3 contains control RNA isolated from C57BL/10 cells treated with 1,000 U ml^{-1} mouse γ-interferon. *b*, S_1 nuclease analysis of cytoplasmic(cyt) and nuclear (nuc) *H-2K*^{b} 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 embryo cells. Lane 1 contains a tRNA control. S_1 nuclease analysis was carried out as described for Fig. 1. All lanes contain an equal amount of RNA (20 μg).

(*hr*771) which produces some E1a protein products only after a delay in permissive cells[10], or with an Ad12 E1a mutant (*in*600) which contains the normal E1a 12S RNA and a truncated 13S RNA[10] resulted in, respectively, no increase (Fig. 2*a*, 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 E1a mutants *hr*751 and *in*600 with each other or with the *hr*1121 E1b mutant resulted in an increase in the level of *H-2K* mRNA (Fig. 2*a*, lanes 4, 3 and 2 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. 2*b*).

Failure of adenovirus E1a mutants to activate the *H-2K*^{b} gene may result from the lack of production of other adenovirus early proteins which are normally activated by the E1a gene products[11,12]. 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

the E1a proteins (ref. 13 and R. Grand and P. Gallimore, personal communication). As shown in Fig. 2*c*, infection with a high titre of E1a mutants (*in*600 and *hr*771) resulted in activation of the $H\text{-}2K^b$ 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 $H\text{-}2K^b$ gene. The E1b 52K protein and the products of the E1a 12S RNA, or 13S exon 1 RNA, are directly or indirectly necessary for $H\text{-}2K^b$ gene activation since high-titre infection with either the E1b 52K mutant (*hr*1121) or the E1a mutant which lacked protein products of both 12S and 13S RNAs (*hr*751) failed to significantly (less than twofold) increase the level of $H\text{-}2K^b$ gene transcripts (Fig. 2*c*). Measurement of the relative rate of transcription of the $H\text{-}2K^b$ 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. 2*d*).

This result may reflect the *trans*-acting enhancing properties of the E1a protein products, which can activate viral[11,12,14], newly introduced[15-18], integrated[19] and endogenous genes[20] either directly or through inactivation of cellular repressor proteins[13,21]. 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 proteins[21,22]; 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 embryo 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 12S 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 antigen[24]. To determine whether other DNA tumour viruses such as SV40 mediate induction of the class I MHC genes using similar mechanisms[25] and whether induction is related to permissiveness of the virus/cell system, we infected mouse embryo cells with either SV40 (non-permissive, as is adenovirus) 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 adenovirus infections, which itself was lower than that induced by interferon in a control experiment (Fig. 3*a*).

Our results are in contrast to those of Schrier *et al.*[5] who reported a decrease in the level of cytoplasmic class I mRNA and suppression of MHC antigen expression in Adl2-, but not in Ad5-infected cells. As Schrier *et al.*[5] 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. 3*b*) 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 labelling of the Ad5- and Ad12-infected cells indicate that the amount of $H\text{-}2K^b$ antigen on the cell surface increases only marginally, if at all, depsite the large increase in cytoplasmic $H\text{-}2K^b$ 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 Ad5 and Ad12, this may not result in an increased expression of MHC antigens on the cell surface because of other viral factors[23]. 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 cells[26-28] alone and/or from cytotoxic T cells[6] as part of an MHC-restricted response[29], is unclear.

This work was supported by the BMRC (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 Ely for gifts of SV40 and polyoma virus, D. Goeddel for mouse γ-interferon, J. Williams for the adenovirus mutants *hr*771 and *hr*1221, 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.

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Irreversible swelling of secretory granules during exocytosis caused by calcium

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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 bilayers[1,2]. Experiments with bilayers demonstrate that phospholipid vesicles must swell if they are to fuse[3]. Also, inhibition of exocytosis in solutions of high osmolarity occurs in several types of secretory cell[4-8]. 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

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