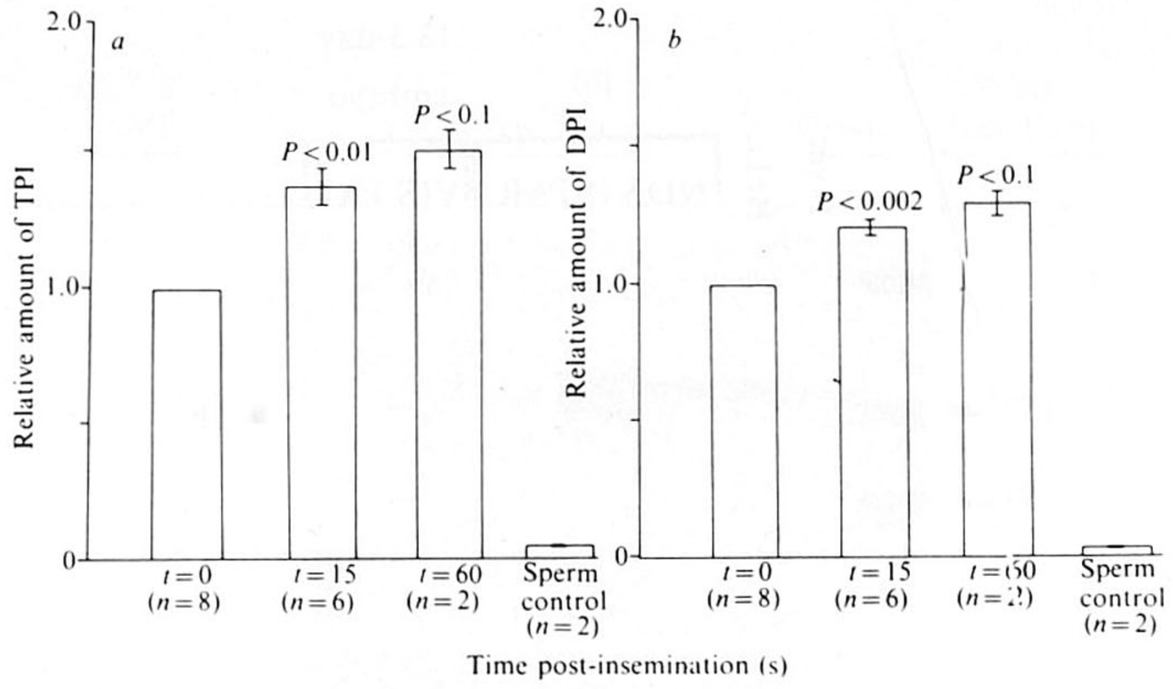

Fig. 2 Changes in the chemical amounts of *a*, TPI and *b*, DPI at various times after fertilization. Data are normalized relative to the amounts of TPI or DPI in the unfertilized eggs ($t = 0$ s) in each experiment. Bars indicate s.e.m., n the number of experiments. P values were determined by Student's t-test; NS, not significant. See text for explanation of sperm control experiments.
Methods: DPI and TPI were purified as described for Fig. 1. Ten ml of a 5% egg suspension was used for each sample. Columns were run at 0 °C. Fractions were dried and the amount of inorganic phosphate in each was determined[28].

Although our results are difficult to interpret without a knowledge of the specific activity of the ATP pool, they did encourage us to assay chemically the amounts of DPI and TPI present in samples of sea urchin eggs taken before and after fertilization (Fig. 2). Using column chromatography as described for Fig. 1, and analysing the phosphate content of the eluted fractions (see Fig. 2 legend), we found that each unfertilized egg contains $2.9 \pm 0.1 \times 10^{-15}$ (s.e.m., $n = 8$) moles of TPI and $6.9 \pm 0.7 \times 10^{-15}$ (s.e.m., $n = 8$) moles of DPI. Increases in amounts of both TPI and DPI are seen 15 s after fertilization, before vesicle exocytosis. At 15 s post-insemination, the amount of TPI is 40% greater than in unfertilized eggs ($P < 0.01$); at 60 s, the increase is 52%. DPI is increased by 22% at 15 s ($P < 0.002$) and by 31% at 60 s.

To exclude the possibility that the sperm used in the inseminations was providing the increased amounts of TPI and DPI seen following fertilization, we extracted these lipids from an amount of sperm equivalent to that used in the fertilization experiments. (The sperm had been exposed to solubilized egg jelly to induce the acrosome reaction—acrosomal processes were seen in 70% of the cells.) The sperm contain both DPI and TPI (see Fig. 2), but the amounts constitute only 9–15% of the changes seen at fertilization.

We conclude that there is an increase in the content of both TPI and DPI following fertilization, but before the onset of vesicle fusion, in the eggs of the sea urchin *S. purpuratus*. Available data from another species of sea urchin[8] indicate that our observed increases in TPI and DPI also precede the rise in intracellular free calcium. These changes in TPI and DPI metabolism might lead to the release of calcium from intracellular stores. If the net increases we report indicate an increased rate of TPI turnover, the egg might be generating inositol-1,4, 5-trisphosphate, a substance known to release calcium from intracellular stores in pancreatic acinar cells[22]. Alternatively, TPI and DPI might contribute to the stimulation of secretion by a direct action occurring in parallel with the rise in free calcium, but not causing it. The presence of negatively charged lipids, and increases in membrane fluidity, both increase the incidence of vesicle fusion with cells[23]. The polyphosphoinositides are highly charged at normal pH—TPI has a net charge of −5, and DPI one of −3 (ref. 24)—which may promote fusion by increasing binding of calcium to the membranes. Moreover, both TPI, and to a lesser extent DPI, increase glycoprotein lateral mobility in the red blood cell membrane[25]. These characteristics of the polyphosphoinositides might be important in initiating cortical vesicle exocytosis.

The increase in polyphosphoinositide content of the sea urchin egg might also be significant with respect to other changes in the egg's metabolism after fertilization[26]. The increase in polyphosphoinositide content that we report precedes all previously reported events, except for the membrane depolarization, and is therefore the earliest known biochemical event following fertilization of the sea urchin egg.

We thank P. Cameron for help with the column chromatography. This work was supported by NIH grants HD-14939 (L.A.J.) and HL-23359 (M.P.S.). M.P.S. is an Established Investigator of the American Heart Association.

Received 30 December 1983; accepted 31 May 1984.

1. Endo, Y. *Expl Cell Res.* **25,** 383–397 (1961).
2. Chandler, D. E. & Heuser, J. *J. Cell Biol.* **83,** 91–108 (1979).
3. Rothschild, L. & Swann, M. M. *J. exp. Biol.* **29,** 469–483 (1952).
4. Paul, M. & Epel, D. *Expl Cell Res.* **65,** 281–288 (1971).
5. Jaffe, L. A., Hagiwara, S. & Kado, R. T. *Devl Biol.* **67,** 243–248 (1978).
6. Schuel, H. *Gamete Res.* **1,** 299–382 (1978).
7. Eisen, A. thesis, Univ. Pennsylvania (1982).
8. Steinhardt, R., Zucker, R. & Schatten, G. *Devl Biol.* **58,** 185–196 (1977).
9. Zucker, R. S. & Steinhardt, R. A. *Biochim. biophys. Acta* **541,** 459–466 (1978).
10. Hamaguchi, Y. & Hiramoto, Y. *Expl Cell Res.* **134,** 171–179 (1981).
11. Baker, P. F. & Whitaker, M. J. *Nature* **276,** 513–515 (1978).
12. Steinhardt, R. A. & Alderton, J. M. *Nature* **295,** 154–155 (1982).
13. Picard, A. & Dorée, M. *Devl Growth Different.* **24,** 155–162 (1982).
14. Whitaker, M. J. & Baker, P. F. *Proc. R. Soc.* B**218,** 397–413 (1983).
15. Billah, M. M. & Lapetina, E. G. *J. biol. Chem.* **257,** 12705–12708 (1982).
16. Weiss, S. J., McKiney, J. S. & Putney, J. W. *Biochem. J.* **206,** 555–560 (1982).
17. Agranoff, B. W., Murthy, P. & Seguin, E. B. *J. biol. Chem.* **258,** 2076–2078 (1983).
18. Perret, B. P., Plantavid, M., Chap, H. & Douste-Blazy, L. *Biochem. biophys. Res. Commun.* **110,** 660–667 (1983).
19. Kinsey, W. H., Decker, G. L. & Lennarz, W. J. *J. Cell Biol.* **87,** 248–254 (1980).
20. Schmell, E. & Lennarz, W. J. *Biochemistry* **13,** 4114–4121 (1974).
21. Chambers, E. L. & Whiteley, A. H. *J. cell. Physiol.* **68,** 289–308 (1966).
22. Streb, H., Irvine, R. F., Berridge, M. J. & Schulz, I. *Nature* **306,** 67–69 (1983).
23. Papahadjopoulos, D., Poste, G. & Schaeffer, B. E. *Biochim. biophys. Acta* **323,** 23–42 (1973).
24. Downes, P. & Michell, R. H. *Cell Calcium* **3,** 467–502 (1982).
25. Sheetz, M. P., Febbroriello, P. & Koppel, D. E. *Nature* **296,** 91–93 (1982).
26. Epel, D. *Curr. Topics dev. Biol.* **12,** 185–246 (1978).
27. Schacht, J. *J. Lipid Res.* **19,** 1063–1067 (1978).
28. Ames, B. N. *Meth. Enzym.* **8,** 115–118 (1966).

Regulated expression of an introduced MHC *H–2K*^{bm1} gene in murine embryonal carcinoma cells

Arnon Rosenthal*†, Stephanie Wright*, Howard Cedar†, Richard Flavell*‡ & Frank Grosveld*

* Laboratory of Gene Structure and Expression, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK
† Department of Molecular Biology, The Hebrew University, Hadassah Medical School, Jerusalem, Israel
‡ Biogen Research Corporation, 14 Cambridge Center, Cambridge, Massachusetts 02142, USA

The transplantation antigens H–2K, H–2D and H–2L are developmentally regulated[1–3], highly polymorphic[4] cell surface proteins encoded by the major histocompatibility gene complex (MHC)[5–7]. First detectable on the early embryo[2,3], they are subsequently expressed on most somatic cells of the adult mouse in association with the protein β_2-microglobulin (β_2M; ref. 5). Cultured F9 embryonal carcinoma (EC) cells can be induced to differentiate along alternative pathways to form either parietal[8] or visceral[9] extra-embryonic endoderm, each concomitant with a change in morphology and pattern of gene expression. Previous reports have demonstrated an increased level of transplantation antigens in differentiated F9 EC cells[10–14], but the cell types expressing them were not defined. Here we show that the level of MHC H–2K^b and β_2M transcripts is increased during both pathways of this differentiation. Expression of a foreign MHC *H–2K*^{bm1} gene was found to be regulated in a similar manner when the gene was introduced into EC cells. In contrast, an introduced rabbit β-globin gene was not regulated but expressed constitutively.

F9 EC cells were differentiated into parietal endoderm by culturing as a monolayer with 10^{-7} M retinoic acid for 14 days[8], or with 10^{-7} M retinoic acid plus 10^{-4} M N^6, O^2-dibutyryl-adenosine 3′,5′-cyclic monophosphate (dbcAMP) plus 10^{-4} μM 3-isobutylmethylxanthine for 5 days, conditions in which 90% of the cells are differentiated[15,16]. For differentiation to visceral endoderm, cells were grown for 6 days as small floating aggregates in bacteriological Petri dishes with 5×10^{-8} M retinoic acid[9]. MHC H–2K^b and β_2M mRNA transcripts were quantitated by S_1 nuclease analysis using an exon–intron and a cDNA probe respectively, which can detect ~10 pg of specific mRNA after densitometric scanning.

Using a 161-nucleotide *Hpa*II fragment spanning part of the β_2M cDNA and adjacent plasmid sequences as a probe for S_1 nuclease protection, no β_2M transcripts were detected in undifferentiated F9 cells (Fig. 1). Differentiation to parietal or visceral endoderm resulted in at least a 20-fold increase in the level of β_2M transcripts, as demonstrated by the presence of 110- and 80-nucleotide fragments resistant to digestion by S_1 nuclease (Fig. 1). This is consistent with the high level of this transcript observed in visceral and parietal endoderm isolated from the extra-embryonic tissues of a 13.5-day-old mouse. The shorter than full-length 80-nucleotide fragment may be due to either a small difference between the cDNA probe and the transcript or cleavage by S_1 nuclease of an A+T-rich region located 85 base pairs (bp) from the labelled *Hpa*II site[17].

S_1 nuclease analysis using a 600-nucleotide *Ava*II exon III–intron probe (Fig. 2*b*) was used to determine the level of H–2K^b mRNA. As judged by the presence of a 230-nucleotide protected fragment, this transcript is produced in very small quantities in undifferentiated F9 cells. After differentiation either to visceral endoderm using retinoic acid or to parietal endoderm using retinoic acid alone or in combination with dbcAMP (Fig. 2*b*), the level of H–2K mRNA increased 5–10-fold as determined by densitometer scanning.

Analysis of RNA samples from other undifferentiated and differentiated F9 cells (given by B. Hogan)[9,15,16], showed levels of H–2K^b mRNAs identical to ours (not shown). Low levels of H–2K^b mRNA in undifferentiated EC cells have been reported previously[18,19] and resemble the results described for laminin. Laminin mRNA is also transcribed at low levels in undifferentiated F9 cells and its transcription is increased following differentiation[15]. The fact that β_2M RNA is not detectable in undifferentiated F9 cells may indicate that the *H–2K* and β_2M genes are regulated differently. Note that despite the low levels of H–2K^b RNA synthesis, H–2K^b antigen might not be detected[10,20] on the cell surface in the absence of β_2M.

Experiments using cell fusion or chromosome transfer between somatic cells and EC cells have shown that the somatic *H–2K* gene is either repressed[21] or remains active in the hybrid cells[19,22,23]. As these experiments were inconclusive, we have used DNA-mediated gene transfer to study the control of H–2K expression.

A 10-kilobase (kb) fragment containing a variant *H–2K^b* gene called *H–2K^{bm1}* was introduced into F9 cells[24]. *H–2K^{bm1}* was covalently linked to Simian virus 40 (SV40) DNA and to the transposon Tn*5*-derived aminoglycosyl phosphotransferase II (AGPT) as a selective marker (Fig. 2*a*). Using the calcium phosphate transformation method[25], 1–10 clones per 5×10^5 cells per μg DNA were obtained. Southern blotting showed that 12 transformants contained 1–15 copies of the introduced DNA per cell (data not shown).

Taking advantage of the fact that the introduced *H–2K^{bm1}* gene and the endogenous *H–2K^b* gene differ in the third exon sequence[24], we prepared a 600-bp *H–2K^b Ava*II probe from this region which allowed us to distinguish between the transcripts from the two genes by S_1 digestion analysis (Fig. 2*b*). Because partial S_1 nuclease digestion of the *H–2K^b Ava*II probe protected by *H–2K^{bm1}* mRNA yields the same fragment as that protected by the *H–2K^b* transcript, we confirmed the results using the corresponding probe isolated from the *H–2K^{bm1}* gene (not shown).

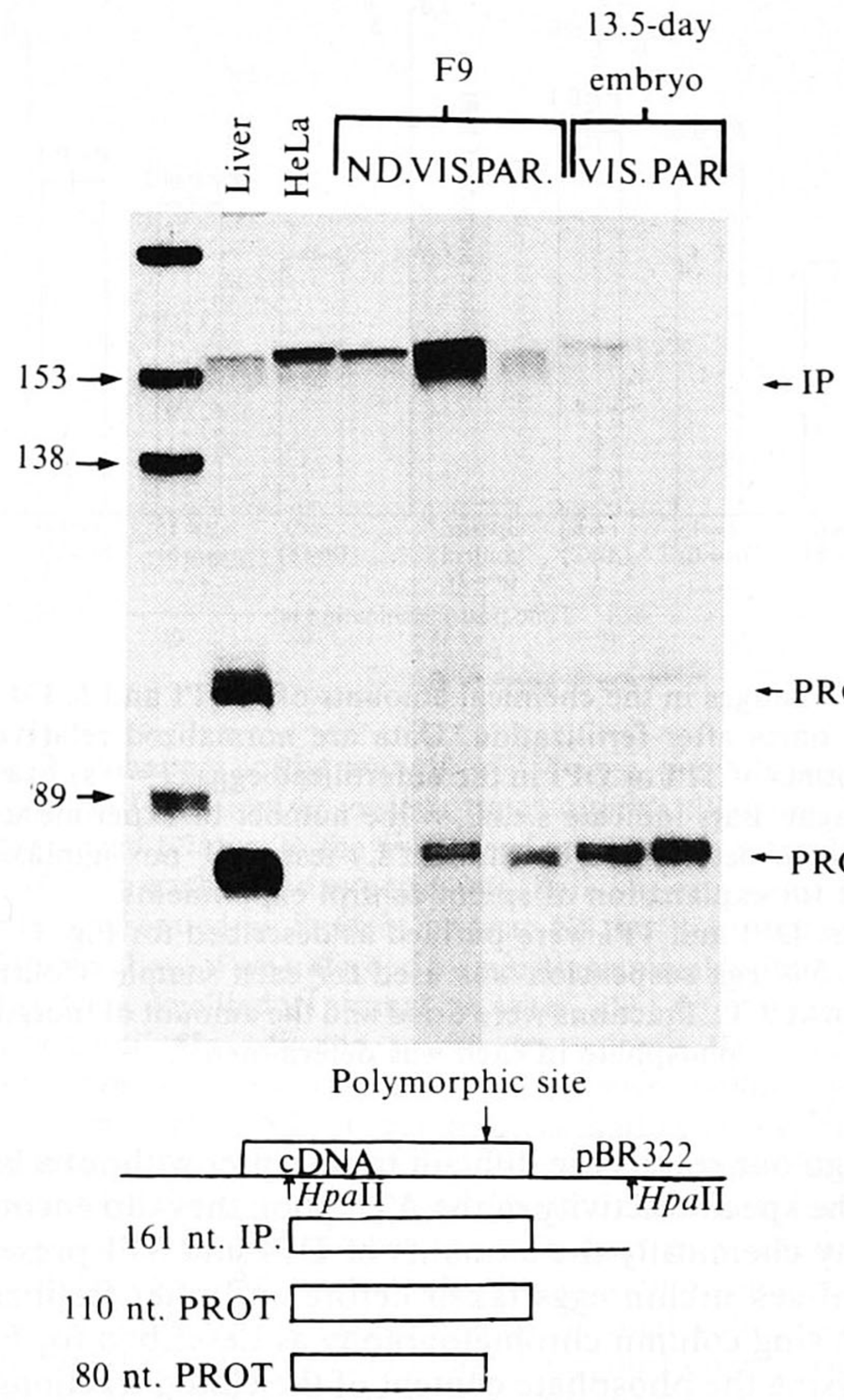

Fig. 1 S_1 nuclease analysis of β_2M mRNA in F9 EC cells before (ND) and after differentiation to parietal (PAR) or visceral (VIS) endoderm. Control mRNA was obtained from visceral and parietal extra-embryonic endoderm of a 13.5-day-old mouse embryo, and from adult mouse liver. Lane 1 contains a radioacivity labelled ΦX × *Rsa* marker.

Methods: A cDNA probe for β_2M (IP) (illustrated at bottom of figure) was end-labelled by reverse transcription, strand-separated and hybridized with total RNA at 37 °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 U of S_1 nuclease (Boehringer) in 300 μl of 300 mM NaAc *p*H 4.8, 200 mM NaCl, 2 mM $ZnSO_4$ at 20 °C for 2 h. S_1-protected DNA (Prot.) was ethanol-precipitated, denatured and electrophoresed on a 7M urea/7% acrylamide gel. F9 lanes: ND, 15 μg of undifferentiated F9 cell RNA; VIS, 3 μg of visceral endoderm F9 cell RNA (overexposed fivefold); PAR, 15 μg of parietal endoderm F9 cell RNA. Embryo lanes: 5 μg of visceral or parietal endoderm (from B. Hogan).

Out of eight clones that contained intact copies of the *H–2K^{bm1}* introduced DNA, seven clones showed a 6–15-fold increase in the level of *H2–K^{bm1}* mRNA on differentiation to parietal endoderm (Fig. 2*b*, clones 2–5). Only one clone expressed the gene in a constitutive manner (not shown). Four clones were shown to contain an incomplete or rearranged copy of the introduced DNA and on differentiation, only the endogeneous *H–2K^b* transcripts could be detected (Fig. 2*b*, clone 1). Note that the level of the endogenous *H–2K^b* mRNA is 5–10-fold lower than the level found in liver and spleen of *H–2K^b* mice. In contrast, the exogenous *H–2K^{bm1}* gene is expressed at higher levels. This effect may be due either to a higher copy number (see legend to Fig. 2*b*) or to selective integration sites.

The increased levels of *H–2K* mRNA observed on differentiation of EC cells could be a result of transcriptional activation or specific stabilization of *H–2K* mRNA; we cannot yet distinguish between these possibilities.

All of the above S_1 analyses were done using a probe which spans one of the exon–intron junctions within the gene. To

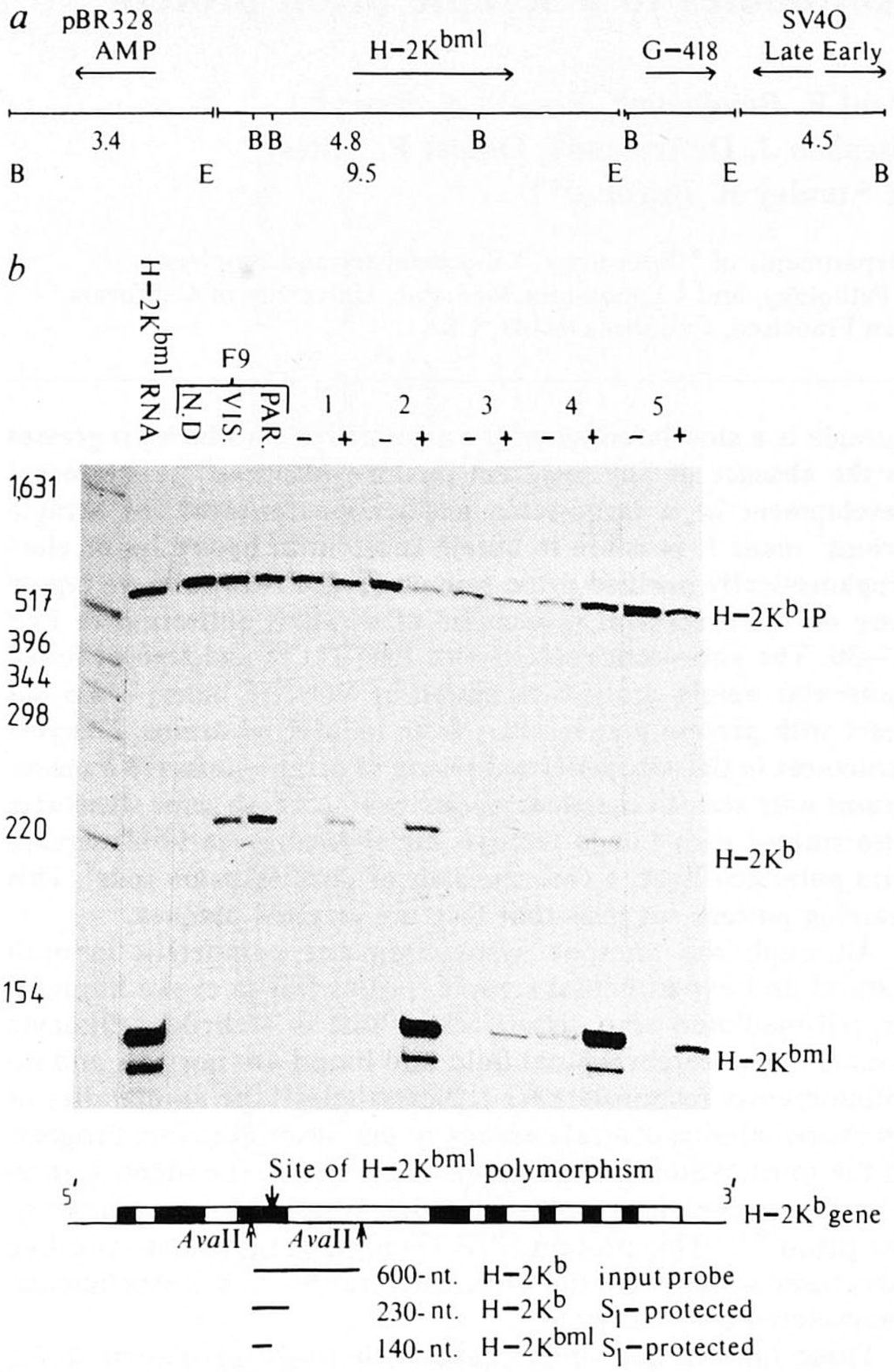

Fig. 2 *a*, Structure of the plasmid introduced into F9 cells. Transcripts are illustrated by arrows in the 5′ to 3′ direction. E and B, *Eco*RI and *Bam*HI sites, respectively. *b*, S_1 nuclease analysis of H–2K^b and H–2K^{bml}, RNA from untransformed (F9) and from *H–2K^{bml}*-transformed (1–5 EC cells) before (−) and after (+) differentiation. Lane labelled H–2K^{bml} RNA, 10 μg of RNA from a transient expression of the *H–2K^{bml}* gene in HeLa cells[31]. F9 lanes: 10 μg of RNA as in Fig. 1. Lanes 1–5, 10 μg of transformed F9 cell RNA containing, respectively, 1–3, 5–8, 3–5, 10–12 and 3–5 integrated copies of the *H–2K^{bml}* gene. *c*, S_1 nuclease analysis of the 5′ end of H–2K mRNA in two F9 transformants, before (−) and after (+) differentiation.

Methods: *b*, A 600-nucleotide intron–exon probe that allowed a distinction to be made between H–2K^{bml} and H–2K^b transcripts (see *a*) was end-labelled with reverse transcriptase, strand-separated and hybridized as in Fig. 1, except that S_1 digestion was for 1 h at 20 °C followed by 1 h at 40 °C. Lane 4 contains a pBR322 × *Hinf* marker. *c*, The 5′ H–2K^b probe was end-labelled with T4 polynucleotide kinase, strand-separated and hybridized as described in Fig. 1.

determine whether these transcripts were properly initiated, we used a probe that covered the 5′ end of the *H–2K^b* and *H–2K^{bml}* genes, which are identical in this region. Although this probe does not distinguish between the mRNA produced from the endogeneous and exogenous genes, it showed only the correct 5′ end expected for H–2K^b and H–2K^{bml} mRNAs, therefore we conclude that transcription of the exogenous gene is correctly initiated (Fig. 2*c*).

In another set of transfection experiments, the rabbit β-globin gene, covalently linked to the same SV40 vector, was introduced into F9 EC cells. S_1 nuclease analysis of four different transformants showed a low level of β-globin mRNA which did not increase following differentiation, while the endogenous H–2K^b mRNA level in the same transformants increased (Fig. 3).

These results suggest that the DNA sequences involved in this induction process must be present on the 10-kb fragment containing the *H–2K^{bml}* gene used in this experiment and that these sequences are specific for the *H–2K* gene. It is highly unlikely that the control sequences would be tissue-specific enhancer elements, as first observed for immunoglobulin genes[26–28]; instead, they may control the activation of the *H–2K* gene at a certain time in the development programme and its subsequent expression in all somatic cells. Induction of this sort may be mediated either by the release of gene suppression of by gene activation and our data are compatible with both of these models.

The fact that visceral-like endoderm and parietal-like endoderm F9 cells, which differ morphologically and functionally, both express H–2K and β_2M may imply that the F9 EC cells originate from embryonal cells predetermined[29] to express transplantation antigens. Thus, although F9 EC cells can be differentiated along alternative pathways[8,9,30], with respect to the expression of MHC genes they may not be different from other, already committed cell systems where increased gene expression can be induced chemically.

This work was supported by the BMRC (UK). A.R. was

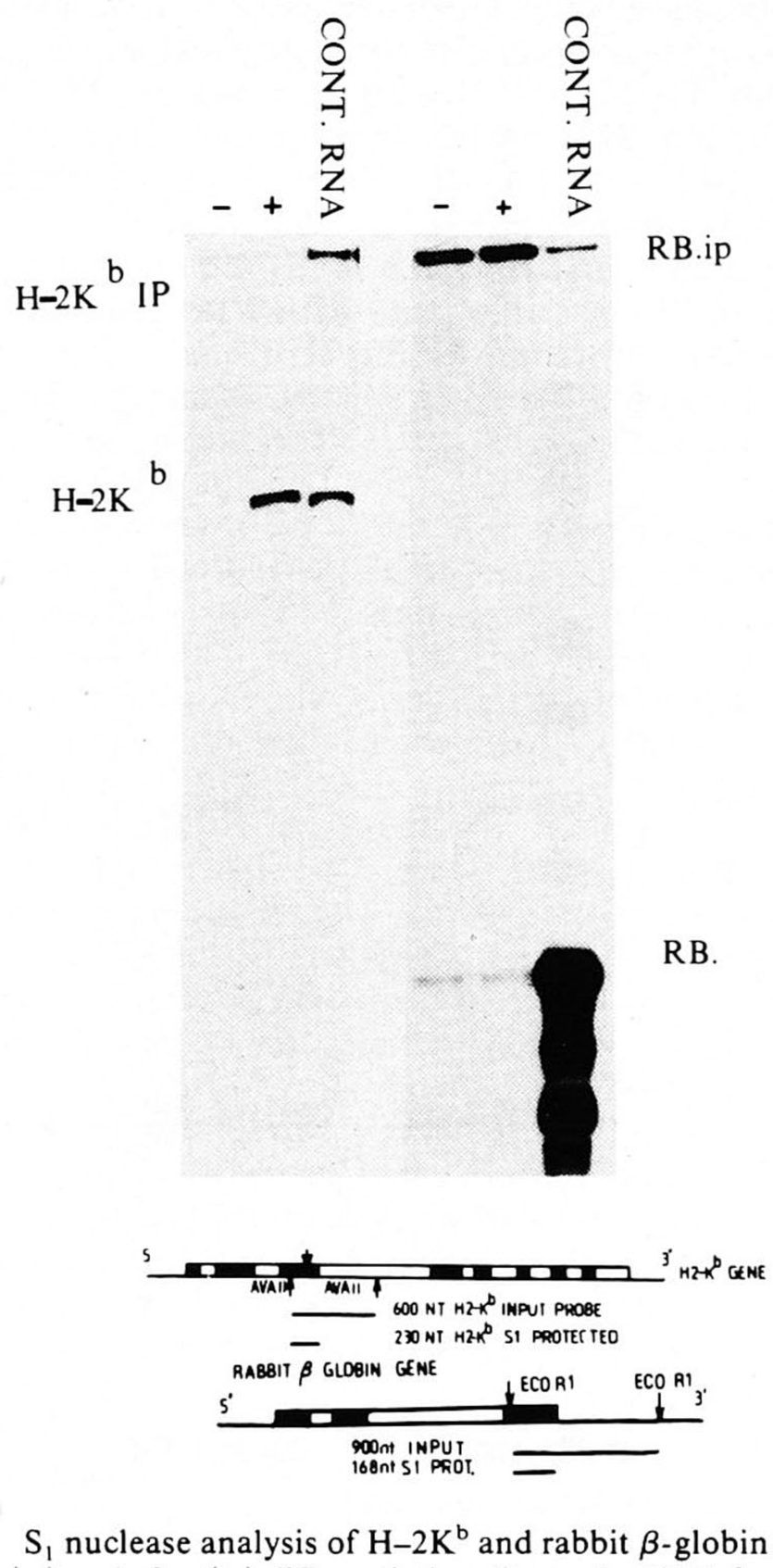

Fig. 3 S_1 nuclease analysis of H–2K^b and rabbit β-globin mRNA before (−) and after (+) differentiation. Control mRNA for H–2K^b was obtained from nontransformed F9 cells after differentiation to parietal endoderm. Control mRNA for rabbit β-globin was obtained from rabbit bone marrow. The 3′ rabbit β-globin probe (RBip) was end-labelled using reverse transcriptase and hybridized as described in Fig. 1 legend.

supported by The Michael and Anna Wix Trust through the Friends of the Hebrew University. We thank D. Barlow and B. Hogan for gifts of RNA and for technical advice, and R. Axel for his hospitality and assistance in the initial transformations.

Received 14 February; accepted 21 May 1984.

1. Strickland, S., Smith, K. K. & Maratti, K. K. *Cell* **21**, 347–355 (1980).
2. Ostrand-Rosenberg, S. *Curr. Topics dev. Biol.* **14**, 147–168 (1980).
3. Webb, C. G. *et al. J. exp. Med.* **146**, 923–932 (1977).
4. Klein, J. *Science* **203**, 516–521 (1977).
5. Ploegh, H. L., Orr, H. T. & Strominger, J. L. *Cell* **24**, 287–299 (1981).
6. Hood, L., Steimmetz, M. & Goodenow, R. *Cell* **28**, 685–687 (1982).
7. Klein, J. *Biology of the Mouse Major Histocompatibility Complex* (Springer, Berlin, 1975).
8. Strickland, S. & Mahdavi, V. *Cell* **15**, 393–403 (1980).
9. Hogan, B. L. & Taylor, A. *Nature* **291**, 235–237 (1981).
10. Nicolas, J. F. *et al. Ann. Microbiol.* **A126**, 3–22 (1975).
11. Croce, C. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 5754–5748 (1981).
12. Knowles, B. B. *et al. Nature* **288**, 615–617 (1980).
13. Jacob, F. *Immunol. Dev.* **33**, 3–32 (1977).
14. Tanaha, K., Appella, E. & Jay, G. *Cell* **35**, 457–465 (1983).
15. Cooper, A. R., Taylor, A. & Hogan, B. L. M. *Devl Biol.* **99**, 510–516 (1983).
16. Kurkinen, M. *et al. Nucleic Acids Res.* **11**, 6200–6209 (1983).
17. Parnes, J. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 2253–2257 (1981).
18. Sherman, M. I. & Miller, R. A. *Devl Biol.* **63**, 27–34 (1978).
19. Benham, P. J., Quintero, M. A. & Goodfellow, P. M. *EMBO. J.* **2**, 1963–1968 (1983).
20. Artzt, K. & Jacob, F. *Transplantation* **17**, 632–625 (1975).
21. Forejt, J. *Cold Spring Harb. Symp. quant. Biol.* **48**, 541–552 (1983).
22. Andrews, D. W. & Goodfellow, P. N. *Somat. Cell Genet.* **6**, 271–284 (1980).
23. Correani, A. & Croce, C. M. *J. cell. Physiol.* **105**, 73–99 (1980).
24. Weiss, E. H. *et al. Nature* **301**, 671–674 (1983).
25. Wigler, G. R. *et al. Cell* **16**, 777–785 (1979).
26. Queen, C. & Baltimore, D. *Cell* **33**, 741–748 (1983).
27. Gillies, S. D. *et al. Cell* **33**, 719–728 (1983).
28. Banerji, J. *et al. Cell* **33**, 729–740 (1983).
29. Murphy, D. *et al. Cell* **35**, 865–871 (1983).
30. Liesi, P. *et al. Nature* **306**, 265–267 (1983).
31. Grosveld, G. C. *et al. Nature* **295**, 120–126 (1982).

Antibodies to a scrapie prion protein

Paul E. Bendheim*, Ronald A. Barry*, Stephen J. DeArmond‡, Daniel P. Stites§ & Stanley B. Prusiner*†

Departments of * Neurology, † Biochemistry and Biophysics, ‡ Pathology, and § Laboratory Medicine, University of California, San Francisco, California 94143, USA

Scrapie is a slow infection of the nervous system which progresses in the absence of any apparent immune response[1–8]. The recent development of a large-scale purification protocol for scrapie prions[9] made it possible to obtain substantial quantities of electrophoretically purified prion protein (PrP 27–30) and we report here on the successful production of a rabbit antiserum to PrP 27–30. The antiserum reacted with PrP 27–30 and several lower molecular weight proteins as shown by Western blots; it did not react with protein preparations from uninfected brains. Discrete structures in the subependymal region of scrapie-infected hamster brains were stained immunocytochemically. These same structures also stained with Congo red dye and showed green birefringence with polarized light, a characteristic of purified prion rods[9]. This staining pattern suggests that they are amyloid plaques.

Although the immune system remains competent in both natural and experimental scrapie, prions fail to evoke humoral or cell-mediated responses[1–8]. The host is afebrile, leukocyte counts in the cerebrospinal fluid and blood are normal, and no inflammatory response is seen. Nevertheless, the animal dies of an overwhelming central nervous system slow infection. Progress in the purification of the scrapie agent[9] led to the identification of a protease-resistant protein which is a major component of the prion[10,11]. This protein (PrP 27–30) aggregates into rod-like structures which have the ultrastructural[12,13] and histochemical characteristics of amyloid[12].

Three rabbits were immunized with 10–20 μg of PrP 27–30, coupled to keyhole limpet haemocyanin (KLH), and boosted twice with 10–20 μg of PrP–KLH. These animals failed to produce antibodies to PrP 27–30. A fourth rabbit immunized with 80 μg of PrP 27–30 without KLH and boosted with 130 μg of PrP 27–30 after 25 days produced antibodies. For subsequent boosts in this rabbit, denatured scrapie prions purified by zonal rotor sucrose gradient sedimentation were used without further electrophoretic purification of PrP 27–30. We estimate that the third and fourth boosts contained 40 μg and 100 μg of PrP 27–30, respectively.

The sera were tested by a modified dot-blot immunoassay[14]. Preimmune serum (Fig. 1, row *a*) did not react with purified PrP 27–30, while only immune serum from the fourth rabbit reacted with purified PrP 27–30 (Fig. 1, rows *b–e*). Reactivity with PrP 27–30 was seen at a 1:1,000 dilution of the serum collected 25 days after the first immunization. Immune sera collected after subsequent boosts showed a progressive rise in titre. After three immunizations, the serum could be diluted 1:20,000 and a positive response was seen with purified PrP 27–30.

Immune serum failed to react with proteins purified from uninfected brains by the same protocol (Fig. 1, rows *f, g*). This antiserum reacted with both denatured and native prions purified from scrapie-infected hamster brains (Fig. 1, rows *h, i*). Preimmune serum at a 1:100 dilution gave a detectable response against the denatured and native prions (data not shown), but failed to react with electrophoretically purified PrP 27–30 (Fig. 1, row *a*). At 1:1,000 dilution, no reaction of the preimmune serum with denatured or native prions could be detected. With antiserum diluted 1:1,000, PrP 27–30 could be detected even after its concentration was reduced by a factor of 100 (Fig. 1, rows *j, k*).

Western blots[15,16] were prepared to confirm that the antiserum was reacting specifically with PrP 27–30 (Fig. 2). PrP 27–30 and