The EMBO Journal vol.3 no.3 pp.597–601, 1984

Functional expression of a mouse H-2K^b gene isolated from non-expressing teratocarcinoma cells

Françoise Daniel-Vedele*, Dominique Morello, Claude Benicourt, Catherine Transy, Odile Le Bail, Fernando Plata[1] and Philippe Kourilsky

Unité de Biologie Moléculaire du Gène, Equipe de Recherche du CNRS no. 201 et Service Commun de l'INSERM no. 20, and [1]Unité d'Immunoparasitologie, Département d'Immunologie, Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cédex 15, France

*To whom reprint requests should be sent

Communicated by P. Kourilsky

Embryonal carcinoma cells do not express H-2 antigens or β2-microglobulin. Recent studies have suggested that the expression of these antigens is likely to be controlled at the level of transcription. To study the precise organization of the corresponding genes and their possible expression in adult mouse cells, we have isolated H-2-related genes from a genomic cosmid library constructed with PCC4-aza-RI from DNA of EC cells. Clones isolated from the library after stringent hybridization with an H-2 cDNA probe were tested for their ability to direct H-2 antigen synthesis after DNA-mediated gene transfer in a fibroblastic L cell. Four clones have been found to code for the major transplantation antigen H-2K^b. Structural analysis showed that these clones contained the same entire H-2K^b gene, identical to the corresponding gene isolated from differentiated C57Bl/10 cells. Furthermore, the present studies showed that this embryonal carcinoma gene was expressed and was functional when transfected into a differentiated cell.

Key words: H-2 gene/molecular cloning/gene transfer/teratocarcinoma cells

Introduction

The major histocompatibility antigens (H-2 antigens) of the mouse consist of polymorphic 44-kd proteins which are encoded by genes in chromosome 17 and are non-covalently associated with β2-microglobulin (β2-m), a 12-kd protein encoded by a gene located on chromosome 2 (for review, see Nathenson *et al.*, 1981; Ploegh *et al.*, 1981). Recent studies using H-2 cDNA probes have shown that, depending on the haplotype studied, the number of H-2-related genes varies between 15 and 50. The majority of these genes have been mapped to the Tla locus and code for less polymorphic differentiation antigens (Hood *et al.*, 1982; Steinmetz *et al.*, 1982; Winoto *et al.*, 1983). The remaining genes have been located within the H-2 complex and code for classical major histocompatibility (MHC) antigens, namely H-2K, H-2D and H-2L. The expression of these latter molecules is developmentally regulated: very early murine embryonic cells as well as embryonal carcinoma (EC) cells, which are widely used to study early embryonic development, do not express MHC antigens. These antigens appear during *in vitro* differentiation of various teratocarcinoma cell lines and are expressed in all adult somatic cells (Webb *et al.*, 1977; Morello *et al.*, 1978).

Previous studies on EC cells have shown that the lack of MHC antigens on their surface is correlated with the absence of significant H-2 and β2-m mRNAs in these cells (Croce *et al.*, 1981; Morello *et al.*, 1982). Studies at the DNA level using Southern blot experiments have revealed that H-2 gene expression is not due to major rearrangements of these genes during development (Steinmetz *et al.*, 1981; Morello *et al.*, 1982). These results have suggested that MHC gene activation is likely to be controlled at the transcriptional level.

To understand the molecular mechanisms of MHC gene regulation, we have constructed a genomic cosmid library with DNA from PCC4-aza-RI, an EC cell line that does not express H-2 antigens. H-2-related genes were isolated after colony hybridization of the library with an H-2 cDNA probe and characterized by analysis of their translated products after transfection into mouse fibroblastic Ltk$^-$ cells. Here, we report the study of four clones coding for the H-2K^b antigen.

Results

Construction and screening of a PCC4-aza-RI DNA library

A cosmid library was constructed from PCC4-aza-RI DNA as previously described (Daniel *et al.*, 1983) (see Materials and methods). This library contained ~200 000 independent clones and was screened under high stringency conditions (see Materials and methods) with an H-2 cDNA probe containing the 3′ moiety of pH-2^d-1 (Brégégère *et al.*, 1981) (Figure 1), which corresponds to a highly conserved region in most H-2-related genes. After transfection in mouse L cells and immunofluorescence testing, four of several positive clones were shown to encode a polypeptide which bound anti-H-2K^b monoclonal antibodies. These clones were further analyzed by restriction mapping and DNA sequencing.

Structural analysis of the K^b region

The Southern blot hybridization of the four cosmid DNAs was performed using as probes three cDNA fragments depicted in Figure 1. Probe a corresponded to the third extracellular domain, the transmembranous and cytoplasmic domains of pH-2^d-1 cDNA clone (Brégégère *et al.*, 1981).

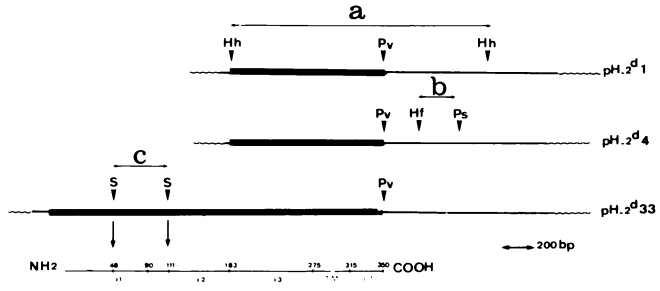

Fig. 1. Schematic diagram of a, b and c H-2 probes. cDNA insertions are drawn as straight lines, flanked by vector sequences. Coding region is indicated by a thick line. The recombinant plasmids are presented in correspondence with the linear structure: α1, α2, α3 represent the three external domains and TM, CT respectively the transmembrane and cytoplasmic regions of the protein. Restriction sites are: Hh = *Hha*I, Pv = *Pvu*II, Hf = *Hinf*I, Ps = *Pst*I, S = *Sac*II.

Probe b was isolated from the pH-2^d-4 cDNA clone (Lalanne *et al.*, 1982) and is specific to the H-2K locus (Xin *et al.*, 1982). Probe b consisted of a 3'-non-coding region of the cDNA. Probe c was a cDNA fragment isolated from clone pH-2^d-33, a H-$2K^d$ cDNA clone (Lalanne *et al.*, 1983). Probe c codes for a peptide extending from the 48th to the 111th amino acid residues.

Restriction maps of the four cosmids were first established using the restriction endonuclease *Kpn*I, as shown in Figure 2. They covered a region of ~65 kb. Two clones, CH2-2 and CH2-6, contained approximately the same genomic DNA region, characterized by the presence of two genes. Each gene is included in an 11-kb *Hind*III fragment; these fragments were subcloned at the *Hind*III site of pAGO (Colbère-Garapin *et al.*, 1979). The resulting recombinant plasmids were used for transfection experiments. Indirect immunofluorescence and radiobinding experiments performed on Ltk$^+$ transformants revealed that only gene 1 (Figure 2) coded for a cell surface antigen recognized by anti-H-$2K^b$ monoclonal antibodies (not shown).

Gene 1 was further analyzed using restriction enzymes individually or in combinations. A detailed map is presented in Figure 2. The 0.8-kb *Kpn*I fragment which hybridized with probe c was subcloned. Partial sequencing showed that this fragment began at the ATG initiation codon and ended within the second domain (112th amino acid). A total of 490 nucleotides was sequenced and compared with the H-$2K^b$ nucleotide sequence recently established by Weiss *et al.* (1983). The only difference between the two sequences was located in the second intron where a CT doublet at position 815 is replaced by a GCT triplet.

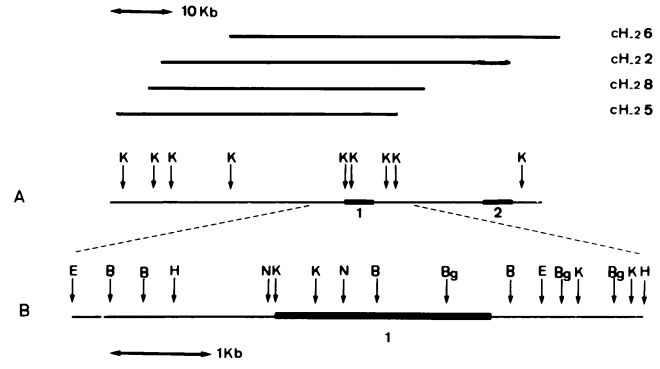

Fig. 2. Restriction endonuclease analysis of the four cosmid clones. The four overlapping clones are represented above the restriction map of the K region **(A)**. Genes 1 and 2 are indicated by thick lines. Restriction maps of gene 1 and its surrounding region is depicted in lane **B**. K = *Kpn*I, E = *Eco*RI, B = *Bam*HI, H = *Hind*III, N = *Nru*I, Bg = *Bgl*II.

Expression of H-2 cosmids in mouse cells

Cosmid DNAs (10 μg) were co-transferred with plasmid pAGO DNA (Colbère-Garapin *et al.*, 1979) (100 ng) containing the herpes simplex virus thymidine kinase (tk) gene, into the fibroblastic Ltk$^-$ cell line using the $CaPO_4$ precipitation procedure (Wigler *et al.*, 1977). Transformants were selected in HAT medium, 48 h after transfection. For each cosmid DNA, uncloned tk$^+$ cells were tested by immunofluorescence analysis. In each case, 70–100% of the cells expressed an H-$2K^b$ antigen at their cell surface. These cells were further subcloned and L clones will be designated as LCH2-2, LCH2-5, LCH2-6, LCH2-8 when transfected by CH2-2, CH2-5, CH2-6 or CH2-8 cosmid DNA, respectively.

Analysis of transformed L cells

Binding of antibodies. The four tk$^+$ clones (LCH2-2, LCH2-5, LCH2-6, LCH2-8) were tested either by indirect immunofluorescence or a ^{125}I-radiobinding assay with five monoclonal antibodies directed against public specificities and two polyclonal antibodies directed against private specificities of H-2 molecules. Results of indirect immunofluorescence analyses are given in Table I: the four tk$^+$ clones were found to be positive with all antibodies which recognize either public or private determinants of the H-$2K^b$ molecule. They were negative with the monoclonal and polyclonal antibodies reactive against the H-$2D^b$ molecule. Expression of the endogenous H-$2K^k$ gene was observed in each case.

Radiobinding assays were performed with the two monoclonal antibodies 20.8.4S and 11.4.1 (Table I) as shown in Figure 3. A Ltk$^+$ clone, transformed with pAGO plasmid DNA alone was used as control. Clones LCH2-2, LCH2-5, LCH2-6 and LCH2-8 bound four times more anti-H-$2K^b$ monoclonal antibody than control Ltk$^+$ cells. In each case, expression of the endogenous H-$2K^k$ gene was slightly decreased, by ~15%.

Cell-mediated cytotoxicity assays. The H-$2K^b$ antigen expressed by LCH2-2 cells was tested for its capacity to function in cell-cell interactions. Anti-H-2^k (BALB/c anti-BALB.K) and anti-H-2^b (C3H anti-BALB.B) cytotoxic T lymphocytes (CTLs) were generated in mixed lymphocyte culture (MLC) and tested for their ability to kill ^{51}Cr-labelled

Table I. Indirect immunofluorescence data on L transformed cells

Serum	Reactivity with H-2 antigens	Immunofluorescence results				
		LCH2-2	LCH2-5	LCH2-6	LCH2-8	Ltk$^+$
Monoclonal 11.4.1 (Ozato *et al.*, 1981)	K^k, K^q, p, r	+	+	+	+	+
Monoclonal 20.8.4S (Ozato and Sachs, 1981)	K^b, D^b, K^d, r, s	+	+	+	+	–
Monoclonal B8.3.24 (Köhler *et al.*, 1981)	K^b (r)	+	+	+	+	–
Monoclonal 28.13.3S (Ozato and Sachs, 1981)	D^b, L^d, q	–	–	–	–	–
Polyclonal H-2.2	D^b (private specificity 2)	–	–	–	–	–
Polyclonal H-2.33	K^b (private specificity 33)	+	+	+	+	–

The two polyclonal antisera are described in Materials and methods. Indirect immunofluorescence assays were performed following the procedure of Pope and Rowe (1964).

L cells. Results reported in Figure 4 (A,B) show that Ltk$^-$ and Ltk$^+$ cells were only killed by anti-H-2^k specific effectors; however, LCH2-2 cells were efficiently killed by both anti-H-2^k and anti-H-2^b CTLs.

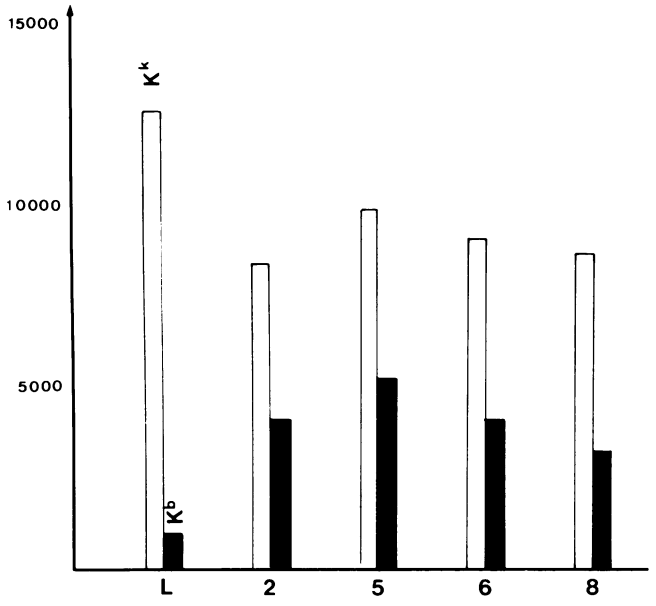

Fig. 3. Histogram of radiobinding assays on transformed L cells. The Y axis represents the number of ^{125}I c.p.m. bound to the cells. White and black boxes correspond respectively to the anti-H-2K^k serum (11.4.1) and the anti-H-2K^b serum (20.8.4S). 2 = LCH2-2, 5 = LCH2-5, 6 = LCH2-6 and 8 = LCH2-8, L = Ltk$^+$ clone (see text).

Intra-H-2 recombinant strains were used to produce monospecific anti-H-2 CTLs: BALB/c anti-BALB.G CTLs were specific for H-2D^b; BALB.G anti-BALB.B CTLs for H-2K^b. As shown in Figure 4 (C,D), LCH2-2 cells were only killed by the anti-H-2K^b CTLs. Control cells, Ltk$^-$ and Ltk$^+$, were not killed by either the anti-H-2D^b or the anti-H-2K^b CTLs.

These data show that the gene product expressed as an H-2K^b molecule at the surface of the LCH2-2 cells is able to function as a target antigen for CTLs; moreover, the exogenous H-2K^b gene product and the endogenous H-2^k gene products were recognized with comparable efficiency.

Discussion

To study the regulation of expression of H-2 genes during differentiation, we have isolated four H-2 cosmid clones from an unamplified genomic library of PCC4-aza-RI EC cell DNA. When transferred into adult mouse L cells, these four cosmid DNAs direct the synthesis of a cell surface molecule which is immunologically identical to a H-2K^b antigen.

The structural analysis of the common gene contained in the four cosmids confirms this serological identification. Restriction maps indicate that the four cosmids cover 65 kb in the H-2K region. Restriction sites are in perfect agreement with the results of Mellor *et al.* (1982) on the H-2K region from C57Bl/10 (H-2^b) liver DNA. Similarly, we could not detect any specific polypeptide synthesis after gene 2 transfer in L cells. Moreover, the partial sequence of the 5′ moiety of our functional gene 1 shows no difference with the H-2K^b

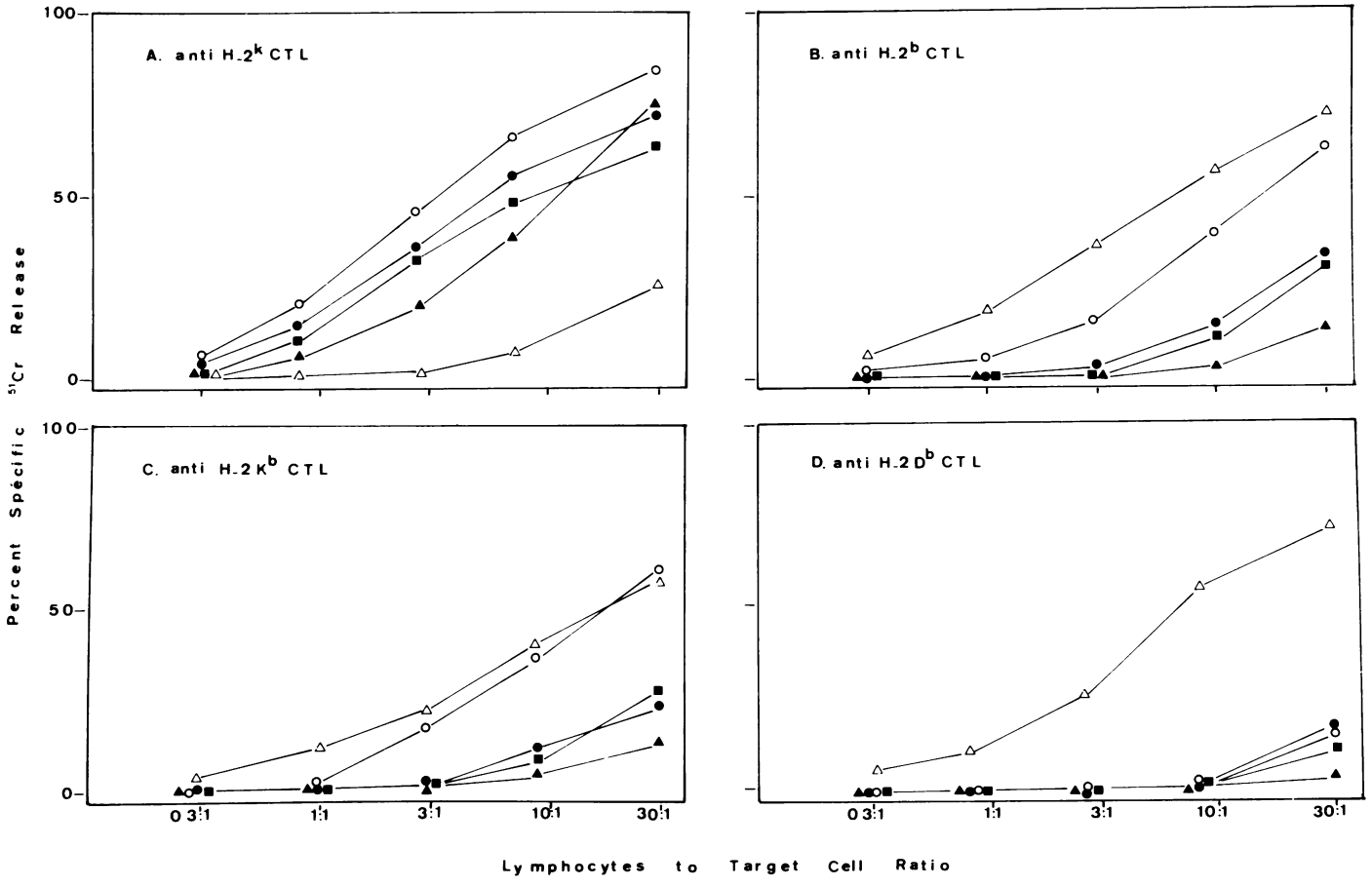

Fig. 4. CTL cell-mediated killing of LCH2-2 cells. Specific cytotoxic T-lymphocyte generation, ^{51}Cr release cytotoxicity assay are described in Materials and methods. The different target cells are represented as follows: △ B.GV (H-2^b); ● Ltk$^-$ (H-2^k); ○ LCH2-2 (H-2^k.H2-K^b); ■ Ltk$^+$ (H-2^k);▲ K.GV S(H-2^k)

gene sequence published by Weiss *et al.* (1983) except a minor one in the second intron. The haplotype of the K region in the 129/Sv strain (H-2^{bc}), from which PCC4-aza-RI has been isolated (Jakob *et al.*, 1973), is the same as that of C57Bl/10. H-2 antigens are highly polymorphic (Klein, 1979) and gene sequences display significant divergence: e.g., comparison of genes coding for H-2K^b and H-2K^d antigens shows 3–4% divergence over coding and non-coding regions (Weiss *et al.*, 1983). 129/Sv and C57Bl/10 strains were separated ~80 years ago (Festing, 1979) but the two K^b gene sequences have so far revealed only one difference. Our results indicate, as suggested by Southern blot analysis (Morello *et al.*, 1982; Steinmetz *et al.*, 1981), that an H-2K^b gene, isolated from non-expressing EC cells, is identical, even in its surrounding regions, to its counterpart in the differentiated cell.

When transferred into adult mouse L cells, this gene directs the synthesis of a normal H-2 antigen, which is recognized by monospecific antibodies directed against private and public specificities of the H-2K^b antigen. Immunofluorescence and radiobinding assays suggest that this molecule presents all the epitopes normally associated with the H-2K^b antigen. Moreover, this molecule is able to function as a target antigen in cell-cell interaction since transformed L cells are not only killed by anti-H-2^k CTL but also by anti-H-2K^b CTL. This functional characteristic of the molecule is reinforced by virus-specific, H-2-restricted CTL killing: LCH2-2 cells transformed with the *gag* gene of Akv-MuLV are killed by BALB.B anti-Gross MuLV CTL (Abastado *et al.*, in preparation). As we have recently shown (Daniel *et al.*, 1983), the β2-m gene isolated from PCC4-aza-RI cell DNA can restore β2-m transient expression, at the surface of β2-m$^-$ lymphoid cells, after gene transfer by protoplast fusion.

Thus, a silent H-2 or β2-m gene isolated from EC cells, can be expressed in a stable or transient manner, after its transfer into an adult fibroblastic or lymphoid cell. Recently, several investigators have obtained expression of specialized genes after transfection into cells of the corresponding specialized type, notably by the β-globin gene in erythroleukaemic cells (Chao *et al.*, 1983) and immunoglobulin genes in myeloma cells (Rice and Baltimore, 1982). In our case, L fibroblastic cells represent a final stage of differentiation which permits expression of H-2 and β2-m genes. These facts suggest that activation of these genes during EC cell differentiation is not due to minor rearrangements at the DNA level in the genes or their surrounding regions. The transcriptional control of H-2 and β2-m gene expression originally proposed by our laboratory and by others (Morello *et al.*, 1982; Croce *et al.*, 1981) must therefore involve mechanisms other than DNA rearrangements.

The chromatin structure of transcribed genes differs from that of inactive genes and consequently, changes in the chromatin configuration apparently precede transcriptional activation of a gene (Weisbrod, 1982). Active chromatin regions are known to be more sensitive to deoxyribonuclease I (Weintraub and Groudine, 1976). However, Croce *et al.* (1982) showed that the silent H-2 genes of teratocarcinoma stem cells are more DNAse I-sensitive than the active genes of the differentiated cells. The modulation of DNA methylation has been implicated as another mechanism of gene control in both viral and cellular models (Razin and Riggs, 1980; Busslinger *et al.*, 1983). Partial de-methylation in the H-2 multigene family occurs during differentiation of EC cells (Morello *et al.*, 1983). Whether this phenomenon involves the H-2K gene and is related to the control of its expression remains to be investigated.

Materials and methods

Mice

The inbred H-2 congenic strains BALB/c (H-2^d), BALB.B (H-2^b), BALB.K (H-2^k) and BALB.G (H-2^g) were used. These mice were bred from breeding pairs provided by Dr. F. Lilly (Albert Einstein College of Medicine, NY), in the animal facilities of the Service d'Immunogénétique de la souris, Hôpital Cochin, Paris. C4H/He mice were bred at the Pasteur Institute from breeding pairs originally provided by the Jackson Laboratories (Maine, USA).

Tumour cells

Continuous leukemia cell lines induced by Gross MuLV were maintained as stationary suspension cultures in RPMI-1640 culture medium supplemented with 10% fetal bovine serum (FBS). B.GV cells were induced in BALB.B (H-2^b) mice and the K.GV cells in BALB.K (H-2^k) mice. PCC4-aza-RI embryonal carcinoma line and its growth conditions are described by Jakob *et al.* (1973).

Antisera

Mouse antisera specific for H-2 antigens were prepared in our laboratory by repeated immunization, as follows. Anti-H-2K^b (H-2.33) serum was obtained from (A x BALB.G)F_1 mice immunized with BALB.B spleen cells; anti-H-2D^b (H-2.2) serum, from BALB/c mice immunized with BALB.G spleen cells.

Isolation of H-2 clones

A cosmid library was constructed following the technique of Grosveld *et al.* (1981). The vector was engineered from plasmid pSV2 (Berg, 1981) by insertion, at the *Eco*RI site, of a 1.8 *Bgl*II cos fragment of pHC79 (Hohn and Collins, 1980). H-2 or H-2-related clones were isolated, using as a probe the *Hha*I fragment of pH-2^d-1 (probe a, Figure 1). This fragment codes for a nearly complete third extracellular domain and the transmembrane and cytoplasmic regions of H-2 antigen (Brégégère *et al.*, 1981). This fragment was labelled with [α-^{32}P]dTTP and [α-^{32}P]dCTP by nick translation to a specific activity in the range of $1-2 \times 10^8$ c.p.m./μg (Rigby *et al.*, 1977).

Nitrocellulose filters carrying lysed bacterial colonies were prepared according to Hanahan and Meselson (1980). Hybridizations were performed for 16 h at 68°C in 1 ml/filter of 2 x SSC, 2 x 10^{-3} M EDTA, 1 x Denhardt solution, potassium phosphate buffer 0.025 M, pH 7.2, 0.5% SDS with 2 x 10^6 c.p.m. of radiolabelled probe/ml. Filters were washed at 68°C in 2 x SSC, 1 x Denhardt solution, 0.5% SDS (4 times, 45 min) and then once in 0.1 SSC for 30 min. Filters were dried and exposed at −70°C using an XAR film (Kodak) with intensifying screens (Dupont de Nemours).

Restriction mapping and sequence analysis

After complete digestion by various enzymes, recombinant cosmid DNAs were run on 0.8% agarose gels and transferred onto nitrocellulose filters according to the Southern procedure (1975). Hybridizations were carried out as above, using 2 x 10^6 c.p.m. of radiolabelled probe in 5 ml of buffer/filter.

DNA sequence was determined according to Maxam and Gilbert (1980) after either 5′ labelling by γ-exchange or 3′ labelling by addition of cordycepin with terminal transferase (Maxam and Gilbert, 1980).

Serological analysis of transformed L cells

Indirect immunofluorescence tests were performed on transformed cells using the conventional procedure (Pope and Rowe, 1964). Cells were allowed to react first with the different antisera (Table I), then with fluorescein immunoconjugated rabbit anti-mouse immunoglobulins (Miles). Cells were studied by immunofluorescence microscopy. Radiobinding assays were performed using the anti-H-2K^b monoclonal antibody 20.8.4S at a dilution of 1/200 and the anti-H-2K^k monoclonal antibody 11.4.1 at a dilution of 1/3000. 200 000 cells per well were incubated in triplicate with the diluted serum, washed three times and incubated with 5 x 10^4 c.p.m. of protein A radiolabelled with ^{125}I. Cells were then washed and counted in a γ scintillation counter.

Lymphocyte cultures and assays

CTL specific for H-2 alloantigens were generated in primary mixed leukocytes cultures (MLC) (Plata *et al.*, 1980). Cell-mediated cytolytic activity was detected with a 3.5 h ^{51}Cr-release cytotoxicity assay as described elsewhere (Plata and Lilly, 1979). Leukemia cells were labelled immediately before the cytotoxicity assay by incubating 2 x 10^6 cells in 0.5 ml serum-free culture medium with 100 μCi $Na_2{}^{51}CrO_4$ at 37°C for 45 min. After labelling, the cells were washed three consecutive times in 10 ml culture medium by centrifugation at 600 *g* for 5 min, and finally resuspended in RMPI-1640 culture medium supplemented with 10% FBS. The cells were counted and distributed in round-bottom microplates at 10^4 cells/well in 100 μl.

L cells were labelled as attached cell monolayers 24 h before the assay, by incubating 2 x 10^6 cells in 1.0 ml serum-free culture medium with 200 μCi $Na_2{}^{51}CrO_4$ at 37°C for 1 h. After labelling, the cell monolayer was rinsed twice with 5 ml culture medium; the cells were trypsinized, washed twice in 10 ml culture medium, and finally resuspended in culture medium supplemented with 10% FBS. Labelled L cells were distributed in flat-bottomed tissue culture microplates at 10^4 cells/well in 200 μl complete culture medium and incubated overnight at 37°C. Immediately before the cytotoxicity assay, culture medium was removed from each well and replaced by 100 μl RPMI-1640 culture medium supplemented with 10% FBS.

Assays were initiated by the addition of cytolytic T lymphocytes at different dilutions in 100 μl culture medium/well. In all cases, spontaneous release values in culture medium alone varied between 10 and 20% of the total incorporated label.

Acknowledgements

We are grateful to Dr. F. Lemonnier, Dr. M. Pierrès and Dr. A.M. Schmitt-Verhulst for their generous gift of monoclonal antibodies, to Dr. F. Colbère-Garapin who provided the Ltk$^-$ clone and the calcium phosphate precipitation procedure. F. Daniel-Vedele is chargée de recherche of INRA. This work was supported by grants of INSERM (SC No. 20), CNRS (ER No. 201) and the Fondation à la Recherche Médicale Française.

References

Berg,P. (1981) *Science (Wash.)* **213**, 296-302.
Busslinger,M., Hurot,J. and Flavell,R.A. (1983) *Cell*, **34**, 197-206.
Brégégère,F., Abastado,J.P., Kvist,S., Rask,L., Lalanne,J.L., Garoff,H., Cami,B., Wiman,K., Larhammar,D., Peterson,P.A., Gachelin,G., Kourilsky,P. and Dobberstein,B. (1981) *Nature*, **292**, 78-81.
Chao,M.V., Mellon,P., Charnay,P., Maniatis,T. and Axel,R. (1983) *Cell*, **32**, 483-493.
Colbère-Garapin,F., Horodniceanu,F., Kourilsky,P. and Garapin,A.C. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3755-3759.
Croce,C.M., Linnenbach,A., Huebner,K., Parnes,J.R., Margulies,D.H., Appella,E. and Seidman,J.G. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 5754-5758.
Daniel,F., Morello,D., Le Bail,O., Chambon,P., Cayre,Y. and Kourilsky,P. (1983) *EMBO J.*, **2**, 1060-1065.
Festing,M.F.W. (1979), *Inbred Strains in Biomedical Research*, published by McMillan Press Ltd.
Grosveld,F.G., Dahl,H.H.M., Boer,E. and Flavell,R.A. (1981) *Gene*, **13**, 227-237.
Hanahan,D. and Meselson,M. (1980) *Gene*, **10**, 63-67.
Hohn,B. and Collins,J. (1980) *Gene*, **11**, 291-298.
Hood,L., Steinmetz,M. and Goodnow,R. (1982) *Cell*, **28**, 685-687.
Jakob,H., Boon,T., Gaillard,J., Nicolas,J.F. and Jacob,F. (1973) *Ann. Microbiol. Inst. Pasteur*, **124B**, 269-282.
Klein,J. (1979) *Science (Wash.)*, **203**, 516-521.
Köhler,G., Fischer-Lindahl,K. and Heusser,L. (1981) in Steinberg,C. and Lefkovits,I. (eds.), *The Immune System*, vol.**2**, Karger, Basel, pp. 202-208.
Lalanne,J.L., Brégégère,F., Delarbre,C., Abastado,J.P., Gachelin,G. and Kourilsky,P. (1982) *Nucleic Acids Res.*, **10**, 1039-1049.
Lalanne,J.L., Delarbre,C., Gachelin,G. and Kourilsky,P. (1983) *Nucleic Acids Res.*, **11**, 1567-1577.
Maxam,A. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499-560.
Mellor,A.L., Golden,L., Weiss,E., Bullman,H., Hurst,J., Simpson,E., James,R.F.L., Townsend,A.R.M., Taylor,P.M., Schmidt,W., Ferluga,J., Leben,L., Santamaria,M., Atfield,G., Festenstein,H. and Flavell,R.A. (1982) *Nature*, **298**, 529-533.
Morello,D., Daniel,F., Baldacci,P., Cayre,Y., Gachelin,G. and Kourilsky,P. (1982) *Nature*, **296**, 260-262.
Morello,D., Gachelin,G., Daniel,F. and Kourilsky,P. (1983) in *Cold Spring Harbor Conf. Cell Prolif.*, **10**, 421-437.
Nathenson,S.G., Uehara,H., Ewenstein,B.M., Kindt,T.K. and Coligan,J.E. (1981) *Annu. Rev. Biochem.*, **50**, 1025-1052.
Ozato,K. and Sachs,D. (1981) *J. Immunol.*, **126**, 317-321.
Ozato,K., Henkart,P., Jensen,C. and Sachs,D. (1981) *J. Immunol.*, **126**, 1780-1785.
Plata,F. and Lilly,F. (1979) *J. Exp. Med.*, **150**, 1174-1186.
Plata,F., Goodenow,M.M. and Lilly,F. (1980) *J. Exp. Med.*, **151**, 726-742.
Ploegh,H.L., Orr,H.T. and Strominger,J.L. (1981) *Cell*, **24**, 287-299.
Pope,J.M. and Rowe,W.P. (1964) *J. Exp. Med.*, **120**, 121-125.
Razin,A. and Riggs,A. (1980) *Science (Wash.)*, **210**, 604-610.
Rice,D. and Baltimore,D. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 7862-7865.
Rigby,P.W.J., Dieckmann,M., Rhodes,C. and Berg,P. (1977) *J. Mol. Biol.*, **113**, 237-251.
Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503-517.
Steinmetz,M., Fzelinger,J.G., Fisher,D., Hunkapiller,T., Pereira,D., Weissman,S.M., Uehara,H., Nathenson,S.G. and Hood,L. (1981) *Cell*, **24**, 125-134.
Steinmetz,M., Winoto,A., Minard,H. and Hood,L. (1982) *Cell*, **28**, 489-498.
Webb,C.G., Gall,E. and Edelman,G.M. (1977) *J. Exp. Med.*, **146**, 923-932.
Weintraub,H. and Groudine,M. (1976) *Science (Wash.)*, **193**, 848-856.
Weisbrod,S. (1982) *Nature*, **297**, 289-295.
Weiss,F., Golden,L., Zakut,R., Mellor,A., Fahrner,K., Kvist,S. and Flavell,R.A. (1983) *EMBO J.*, **2**, 453-462.
Wigler,M., Silverstein,S., Lee,L.S., Pellicer,A., Chang,Y.C. and Axel,R. (1977) *Cell*, **11**, 223-232.
Winoto,A., Steinmetz,M. and Hood,L. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 3425-3429.
Xin,J.H., Kvist,A. and Dobberstein,B. (1982) *EMBO J.*, **1**, 467-471.

Received on 15 December 1983