# Structure and expression of the mouse β2-microglobulin gene isolated from somatic and non-expressing teratocarcinoma cells

Françoise Daniel*, Dominique Morello, Odile Le Bail, Philippe Chambon, Yvon Cayre and Philippe Kourilsky

Unité de Biologie Moléculaire du Gène, E.R. C.N.R.S. 201 and S.C. I.N.S.E.R.M. 20, Institut Pasteur, 25, rue du Dr. Roux, 75724 Paris Cédex 15, France

Mouse teratocarcinoma cells express neither H-2 heavy chains nor β2-microglobulin (β2-m). We have constructed two genomic libraries, one from PCC4-aza-RI embryonal carcinoma cells and the other from their adult syngenic counterpart 129/Sv liver cells (H-2$^{bc}$). The libraries were screened with a full length mouse β2-m cDNA probe which we isolated and sequenced. Two cosmid clones carrying the entire β2-m gene were isolated, one from each library. There was no detectable difference in structure between the two genes. Furthermore, both were shown to be active and to restore β2-m synthesis upon transfer into mutant cells deficient in β2-m. Irreversible DNA alterations in or around the β2-m gene are thus unlikely to account for the lack of β2-m gene expression in embryonal teratocarcinoma cells.
Key words: β2-microglobulin/gene transfer/molecular cloning/teratocarcinoma cells

## Introduction

Mouse β2-microglobulin (β2-m) is a polypeptide 99 amino acids long found associated with a variety of membrane glycoproteins which include the class I transplantation antigens. β2-m, as well as H-2 antigens, is absent from the fertilized egg, but clearly detectable at the surface of day 7 embryos (Buc-Caron et al., 1978). However, it has been reported to be present in stage two cell embryos (Sawicki et al., 1981). β2-m is serologically undetectable in embryonal carcinoma cells (EC cells) (Dubois et al., 1976; Morello, 1978). There is no accumulation of H-2 and/or β2-m poly(A)$^+$ RNA either in the cytoplasm (Croce et al., 1981; Morello et al., 1982) or in the nucleus of EC cells (Morello et al., 1983). When differentiation of EC cells is induced in vitro, it appears (like H-2) in increasing amounts on an increasing number of cells (reviewed in Gachelin, 1978). To analyze whether the β2-m gene isolated from EC cells can be expressed upon introduction into adult cells, we have cloned it from a cosmid library prepared with DNA of PCC4-aza-RI EC cells. As a control, we also isolated the β2-m gene from a library of 129/Sv liver DNA. We show that genes from both sources are structurally indistinguishable and both can be expressed upon transfer into β2-m$^-$ mutant cells.

## Results

### Isolation of a mouse β2-m cDNA probe

A cDNA library, constructed in plasmid pBR327 from poly(A)$^+$ RNA extracted from DBA/2 mouse liver (Lalanne et al., 1983) was screened by in situ hybridization with a human β2-m cDNA probe kindly provided by S.Suggs (Suggs

*To whom reprint requests should be sent.

© IRL Press Limited, Oxford, England.

et al., 1981). Four out of ~2000 clones were found to hybridize under low stringency conditions (see Materials and methods). The one which carried the longest insert, pβ2-m2, was further studied and sequenced. The restriction map and the strategy used to determine the nucleotide sequence are shown in Figure 1. The sequence (711 bp) is indicated in Figure 2. pβ2-m2 contains the entire β2-m coding sequence including the signal sequence, 52 bp upstream of the ATG and 238 bp of the 3' non-coding region followed by a poly(A) stretch of 64 residues. There is identity with the sequence of exons in the β2-m gene described by Parnes and Seidman (1982), except at position −3 of the amino acid sequence where we found a TTG instead of CTG, both triplets coding for leucine. Therefore, our data confirm the existence of a signal peptide of 20 amino acids with Met in position −20 and Ala in position −19 rather than Ser, as reported by Lingappa et al. (1979). Mapping of the promoter of the β2-m gene has not yet been reported. Unless a very long 5'-untranslated region exists, pβ2-m2 is likely to be a full length or nearly full length cDNA clone, but this cannot be ascertained. The 3'-untranslated region contains two polyadenylation signals (AATAAA, underlined in Figure 2). The β2-m gene is known to be transcribed into (at least) two major RNA species (Croce et al., 1981), ~800 and 1000 nucleotides long (Morello et al., 1982) but it is not yet possible to correlate precisely pβ2-m2 with either of these transcripts.

The 85th amino acid underlined in the sequence (Figure 2) is Asp, coded by a GAC triplet. pβ2-m2, therefore, encodes the more acidic form of β2-m (β2-m$^a$), while the more basic form (β2-m$^b$) is specified by an Ala residue (Gates et al., 1981). This allelism correlates with the existence (b form) or the absence (a form) of a BglI restriction site in the gene (our unpublished results). The PstI-PstI and PstI-EcoRI fragments which served as 3'- (probe A) and 5'- (probe B) specific probes, respectively, are shown in Figure 1. Probe C was a mixture of the two 350-bp PstI-PstI fragments encompassing the entire insert.

### Isolation of the β2-m gene from PCC4-aza-RI and 129/Sv liver libraries

A cosmid vector was engineered from plasmid pSV2 (Berg,



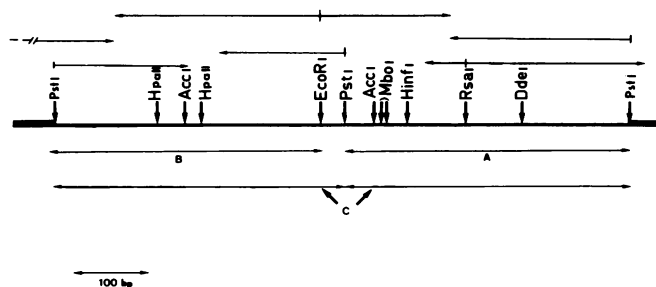Fig. 1. Restriction endonuclease analysis of pβ2-m2. pBR327 vector DNA is represented as a thick line. The strategy used to determine the nucleotide sequence of pβ2-m2 is described above the restriction map. Arrows indicate the sequenced fragments, tails representing the 5' or 3' labelled ends. Fragments A, B and C below the restriction map were used as probe in in situ hybridizations and Southern blotting experiments.

```
                                                                    M   A   R   S   V   T   L   V   F   L   V   L   V   S
                  ATTTCAGTGGCTGCTACTCGGCGCTTCAGTCGCGGTCGCTTCAGTCGTCAGC ATG GCT CGC TCG GTG ACC CTA GTC TTT CTG GTG CTT GTC TCA
                                                                   -20

    L   T   G   L   Y   A   I   Q   K   T   P   Q   I   Q   V   Y   S   R   H   P   P   E   M   G   K   P   M   I   L   M   C
    CTG ACC GGC TTG TAT GCT ATC CAG AAA ACC CCT CAA ATT CAA GTA TAC TCA CGC CAC CCA CCG GAG AAT GGG AAG CCG AAC ATA CTG AAC TGC
                        1                                                                   20

    Y   V   T   Q   F   H   P   P   H   I   E   I   Q   M   L   K   M   G   K   K   I   P   K   V   E   M   S   D   M   S   F
    TAC GTA ACA CAG TTC CAC CCG CCT CAC ATT GAA ATC CAA ATG CTG AAG AAC GGG AAA AAA ATT CCT AAA GTA GAG ATG TCA GAT ATG TCC TTC
                                            40

    S   K   D   W   S   F   Y   I   L   A   H   T   E   F   T   P   T   E   T   D   T   Y   A   C   R   V   K   H   D   S   M
    AGC AAG GAC TGG TCT TTC TAT ATC CTG GCT CAC ACT GAA TTC ACC CCC ACT GAG ACT GAT ACA TAC GCC TGC AGA GTT AAG CAT GAC AGT ATG
                    60                                                                   80

    A   E   P   K   T   V   Y   W   D   R   D   M
    GCC GAG CCC AAG ACC GTC TAC TGG GAT CGA GAC ATG TGATCAAGCATCATGATGCTCTGAAGATTCATTTGAACCTGCTTAATTACAAATCCAGTTTCTAATATGCTATAC
                                            99


    AATTTATGCACGCAGAAAGAAATAGCAATGTACACATCACCTTCTTTATATCTTACTTTAAATGTTTTATGCATGTTTTCAAAAATTGGAAATATCCTAGATAGCTGAGCAATAAATCTTCAA


    TAAGTATTTTGATCAGAATAATAAATATAATTTTAAGAACAAAAAAAAAAAAAAAA.................
```

**Fig. 2.** Nucleotide sequence of pβ2-m2. The coding sequence is represented in triplets according to the genetic code. Letters represent the corresponding amino acids. Negative numbers are used for the signal peptide. The 85th amino acid characteristic of the β2-m$^a$ allele and the two polyadenylation signals are underlined.
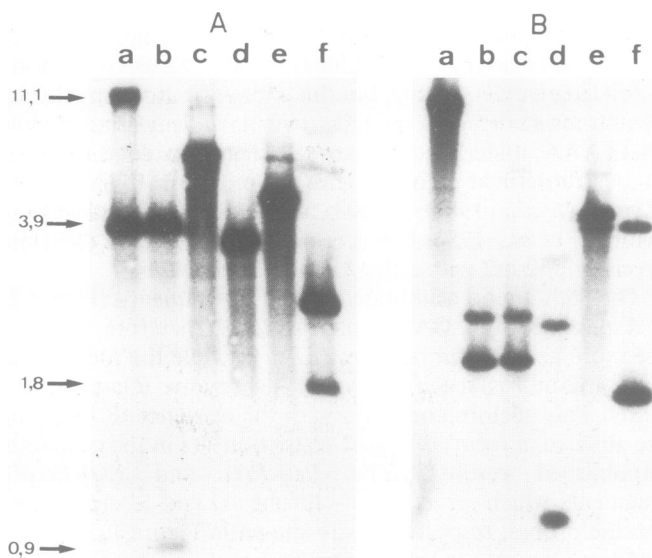


**Fig. 3.** Southern blot hybridization of cβ2-m1 DNA with 3' (A) and 5' (B) pβ2-m2 probes. Hybridizations were performed as described in Materials and methods. cβ2-m1 DNA was digested by: lane a: *Bam*HI; lane b: *Bam*HI and *Eco*RI; lane c: *Eco*RI; lane d: *Eco*RI and *Hind*III; lane e: *Hind*III; lane f: *Hind*III and *Bam*HI. Sizes in kb (within 10% error) shown in the margins were determined from stained *Hind*III digests of phage λS7 DNA.
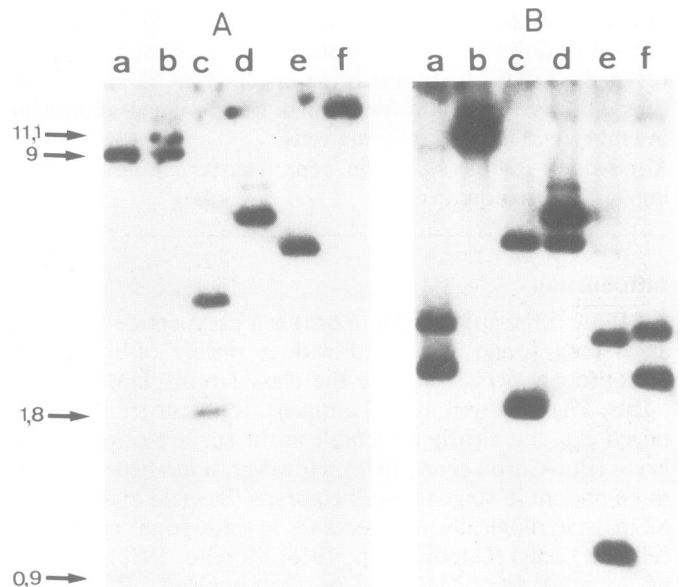


**Fig. 4.** Southern blot hybridization of cβ2-m2 DNA with 3' (A) and 5' (B) pβ2-m2 probes. Experiments and size determinations were performed as described in Figure 3 except that the cβ2-m2 DNA was digested from the left to the right, by: lane a: *Eco*RI and *Bam*HI; lane b: *Bam*HI; lane c: *Hind*III and *Bam*HI; lane d: *Hind*III; lane e: *Eco*RI and *Hind*III; lane f: *Eco*RI.

1981) as outlined in Materials and methods, and kindly provided by J.Papamatheakis. It is 7 kb long and carries the *Ecogpt* gene. Following the method of Grosveld *et al.* (1981), we built two genomic libraries containing large inserts (30–40 kb) of PCC4-aza-RI and 129/Sv liver DNA. Each library contained ~200 000 independent clones and was screened with probe C (Figure 1). One positive recombinant cosmid (cβ2-m1) was isolated from the PCC4-aza-RI library, and another one (cβ2-m2) from the liver library.

*Kpn*I was convenient to establish the overall restriction maps of purified cβ2-m1 and cβ2-m2 DNAs. Three restriction enzymes (*Bam*HI, *Eco*RI, *Hind*III) were also used alone or in combinations. Southern blotting experiments were performed (Figures 3 and 4), using the 3' probe (A) and the 5'

probe (B) to elaborate the restriction maps of the two genes and of their surrounding regions (Figure 5). The inserts in cβ2-m1 and cβ2-m2 are 35 kb and 39 kb long, respectively, within <10% error. Both contain an entire β2-m gene. Their restriction maps with *Bam*HI, *Eco*RI, *Hind*III and *Kpn*I in and around the gene, are identical and fit perfectly the map of the β2-m gene established by Parnes and Seidman (1982), except that the *Hind*III site, at the 3' end of the gene, is, in our case, located 200 bp closer to exon 4. Thus, the β2-m gene in non-expressing PCC4-aza-RI cells has a structure similar, or identical, to that in liver cells. This conclusion is further strengthened by the visualization of heteroduplexes between restriction fragments of cβ2-m1 and cβ2-m2 under the electron microscope which revealed no difference in several kilo-

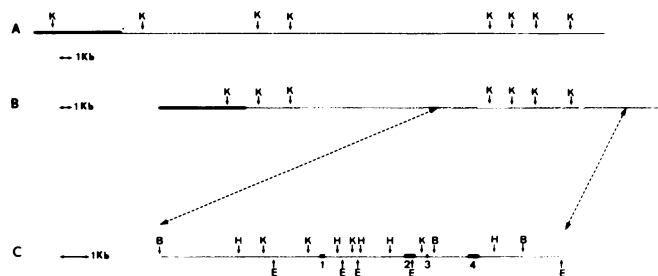**Fig. 5.** Restriction endonuclease analysis of cβ2-m2 (**A**) and cβ2-m1 (**B**) using *Kpn*I restriction enzyme. Thick lines represent vector DNA. Restriction endonuclease analysis of β2-m gene and surrounding region (**C**): K = *Kpn*I; B = *Bam*HI; H = *Hind*III; E = *Eco*RI. Black boxes 1-2-3-4 define the regions of β2-m genes represented in mature mRNA (as defined by Parnes and Seidman, 1982).



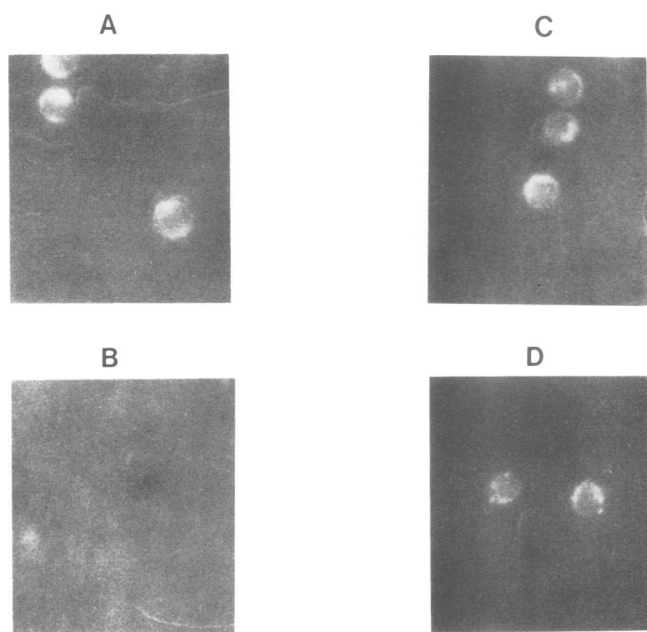**Fig. 6.** Fluorescence micrographs of parental and transformed RI(β2-m⁻) cells tested with rabbit anti-β2-m antiserum and fluorescein conjugated goat anti-rabbit. **A** = parental RI cells, **B** = RI(β2-m⁻) cells, **C** = RI(β2-m⁻) transformed with cβ2-m1, **D** = RI(β2-m⁻) transformed with cβ2-m2.

bases at the 5' end of the gene (data not shown).

### Transient expression of the cloned β2-m gene in RI(β2-m⁻) mouse cells

We undertook to transfer the two genes into appropriate recipient cells to examine if they are both functional. Since we had no reagent to detect specifically a β2-mᵃ polypeptide in a β2-mᵇ background, we could not introduce our β2-mᵃ genes into β2-mᵇ cells. We, therefore, turned to mutant cells deficient in the synthesis of β2-m. One such cell line, named RI(β2-m⁻) was isolated by immunoselection as a TL⁻, derivative of a C58 thymoma (Hyman and Stallings, 1976). Parnes and Seidman (1982) demonstrated that these cells show alterations in the β2-m gene of both chromosomes.

To introduce cβ2-m1 and cβ2-m2 DNA into these cells, we devised a modification of the protoplast fusion technique described by Rassoulzadegan *et al.* (1982), as explained in Materials and methods. The expression of β2-m at the cell surface was monitored by an indirect immunofluorescence assay, using a rabbit anti-mouse β2-m serum adsorbed on

**Table I.** Expression of β2-m in parental RI and RI(β2-m⁻) cells transfected either with cβ2-m1 or cβ2-m2.

| Cell line | Mean fluorescence | % |
|---|---|---|
| R1 | 336 | 100 |
|  | 422 | 77 |
|  | 577 | 42 |
| R1 (β2⁻) | 161 | 100 |
|  | 201 | 70 |
|  | 602 | 5 |
| R1 (β2⁻) cβ2-m1 | 272 | 100 |
|  | 429 | 58 |
|  | 747 | 25 |
| R1 (β2⁻) cβ2-m2 | 309 | 100 |
|  | 471 | 61 |
|  | 762 | 30 |

For each selected window (see Materials and methods), the mean fluorescence and percentage of corresponding fluorescence cells were calculated. Fusion of RI(β2-m⁻) cells with a cosmid carrying an H-2 gene (to be published) give results which are identical to the RI(β2-m⁻) cells results (data not shown).

Daudi cells. Observation under the fluorescence microscope, 72 h after protoplast fusion, showed that both cosmids restore β2-m expression in the RI(β2-m⁻) cells (Figure 6). These results were quantitated by use of a fluorescence activated cell sorter. About 50% of the cells expressed β2-m at the surface with a fluorescence intensity identical to that of the RI(β2-m⁺) parental cell line and several fold higher than that of the RI(β2-m⁻) parental cell line. About 20% of the transformed cells showed a fluorescence intensity higher than that of the RI(β2-m⁺) cell line (Table I).

### Discussion

Here we describe the isolation and sequence of a β2-m cDNA clone from DBA/2 liver mRNA which is likely to be full length or nearly so, and encodes the more acidic form of β2-m. We used it as a probe to clone the β2-m gene from two sources: PCC4-aza-RI cells, which do not express β2-m and H-2, and their adult syngenic counterpart, 129/Sv liver cells. The genomic libraries contained ~200 000 clones each, i.e., more than one genome equivalent. We found only one β2-m gene in each library and no β2-m related gene or β2-m pseudogene. Thus, our results are compatible with the existence of a single β2-m gene per haploid genome, a conclusion reached by Parnes and Seidman (1982) from the analysis of the genetic defects of the RI(β2-m⁻) cell line.

Our two cosmid clones, cβ2-m1 and cβ2-m2 show identical restriction maps in and around the β2-m gene, as judged from restriction analysis and Southern hybridization with 5' and 3' specific probes, but discrete structural differences between the β2-m gene isolated from EC cells and adult cells would not have been detected. We, therefore, proceeded to show that both genes are active. The experiment required gene transfer into a lymphoid cell line, which had not been achieved before, and which we performed through a modification of the protoplast fusion technique. β2-m expression was detected with both genes, more reliably 72 h rather than 48 h after fusion. β2-m was less easily detected and in a smaller population of transformed cells than SV40 T antigen in control experiments (Y.Cayre and B.Cami, unpublished data). Many factors could interfere with β2-m expression and/or detection. Protoplast fusion for the transfer of cosmid DNA from

a *recA⁻ Escherichia coli* host may be less efficient. The synthesis and/or transport of β2-m to the membrane may depend on the cellular cycle or the physical state of the membrane altered by the fusion, or be limited by the synthesis of the presently unidentified heavy chain presumably associated with it.

Together, the finding that the β2-m gene extracted from EC cells has a structure similar to that extracted from adult cells, and the demonstration that it can be expressed in β2-m⁻ mutant cells, argue very strongly that there is no DNA rearrangement associated with its activation *in vivo*. Other structural modifications may, however, take place, such as partial demethylation, which we know occurs in the case of H-2 genes (Morello *et al.*, 1983). Further analyses on the structure and expression of the β2-m gene transferred into EC cells should shed some light on the developmentally controlled expression of the β2-m gene.

## Materials and methods

### Materials

Bacterial strains *E. coli* BHB 2688 and BHB 2690 (Hohn, 1980) were used for preparation of the lambda packaging mix. Packaged cosmid DNA was transfected and propagated in *E. coli* 1046 (803 rk⁻mk⁻ su3⁺ recA⁻) (Murray *et al.*, 1977).

PCC4-aza-R1 and its growth conditions are described in Jakob *et al.* (1973). R1 and RI (β2-m⁻) (Hyman and Stallings, 1976) cells were grown in RPMI 1640 medium (Gibco) supplemented with glutamine (2 mM) and 10% fetal calf serum. Restriction endonucleases were from Boehringer Mannheim, nitrocellulose filters BA 85 from Schleicher and Schüll. [α-³²P]nucleotides (2000−3000 Ci/mmol), [γ-³²P]nucleotides (2000 Ci/mmol) and cordycepin (5000 Ci/mmol) were purchased from Amersham. T4 nucleotide kinase was from P.L. Biochemicals and deoxynucleotidyl terminal transferase from B.R.L.

Rabbit anti-β2-m antiserum (Natori *et al.*, 1974), a kind gift from Dr.Tanigaki, was absorbed on Daudi cells.

### Isolation and sequence of pβ2-m2

Poly(A)⁺ RNA was extracted from DBA/2 mouse liver and complementary cDNAs were cloned as described by Lalanne *et al.* (1983). *In situ* hybridizations were performed for 16 h at 60°C in 1 ml per filter of 2 x SSC, 2 x 10⁻³ M EDTA 1 x Denhardt solution, potassium phosphate buffer pH 7.2, 0.025 M, 0.5% SDS with 2 x 10⁶ c.p.m. of radiolabelled *Pst*I fragment of human β2-m cDNA (Suggs *et al.*, 1981). Filters were washed at 60°C in 1 x SSC and 0.5% SDS (4 times 45 min). pβ2-m2 was analyzed by restriction mapping and its 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).

### Construction of cosmid libraries

High mol. wt. DNA was extracted from PCC4-aza-R1 cells and mouse 129 liver as described by Gross-Bellard *et al.* (1973). These two DNAs were partially digested by *Sau*3A and fractionated in a sucrose gradient according to Grosveld *et al.* (1981). The size of the DNA in each fraction was measured in a 0.2% agarose gel. Fractions of ~ 35 kb were used for ligation.

The cosmid vector, pSV2cos, was constructed by J.Papamatheakis (unpublished work) as follows: pHC79 DNA (Hohn and Collins, 1980) was digested by *Bgl*II. The 1.8-kb fragment containing the cos site was recovered from a 1% agarose gel. pSV2 DNA (Berg, 1981) was digested by *Eco*RI and treated with bovine alkaline phosphatase (Worthington). Both DNAs were treated with the Klenow enzyme (Boehringer Mannheim) and ligated. pSV2cos was recovered after *in situ* hybridization using the 'cos' fragment of pHC79 as a probe.

Vector and genomic DNAs were ligated and packaged *in vitro* as described by Grosveld *et al.* (1981).

### In situ hybridization − Southern blotting

DNA fragments used as probes were recovered from low gelling agarose gels (Wieslander, 1979). They were labelled with [α-³²P]dTTP and [α-³²P]dCTP by nick translation to a specific activity in the range of 1−2 x 10⁸ 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 per 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 10⁶ c.p.m. of radiolabelled probe per 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 x SSC for 30 min. Filters were dried and exposed at − 70°C using a XAR film (Kodak) with intensifying screens (Dupont de Nemours). After complete digestion by various enzymes, recombinant cosmid DNAs were run on 0.8% agarose gels and transferred to nitrocellulose filters according to Southern (1975). Hybridizations were carried out, as above, using 2 x 10⁶ c.p.m. of radiolabelled probe in 5 ml of buffer per filter.

### Protoplast fusion

Protoplasts were prepared according to the procedure of Rassoulzadegan *et al.* (1982) with some modifications. *E. coli* bacteria (strain 1046) carrying the cosmid recombinants were grown up in L broth to a density of 2 x 10⁸ cells/ml. (In our hands, chloramphenicol amplification was not required.) 50 ml of culture were centrifuged, resuspended in 500 μl of 50 mM Tris-HCl, pH 8.0, containing 20% sucrose and allowed to stay on ice for 10 min. 100 μl of a freshly made solution of lysozyme (5 mg/ml) (Sigma) were added and the suspension was incubated for 10 min at 0°C. 250 μl of 0.25 M EDTA, pH 8.0, were added and the conversion of bacteria into protoplasts was followed under the microscope. The suspension was then diluted by addition of 750 μl of 50 mM Tris-HCl, pH 8.0, containing 7% sucrose.

Protoplasts were centrifuged at 2000 r.p.m. during 10 min. At the same time, 5 x 10⁶ RI(β2-m⁻) cells were washed with RPMI 1640 medium without fetal calf serum and collected by centrifugation. Protoplasts were resuspended in 1 ml of a 41.7% PEG 1500 solution (20 g of PEG 1500 dissolved in 28 ml of Dulbecco's phosphate buffered saline 1 x containing 15% dimethylsulfate) and this suspension was added on the RI(β2-m⁻) cells pellet, mixed gently during 1 min at room temperature. 10 ml of RPMI 1640 without fetal calf serum were added slowly (during ~ 5 min), the cells were then washed and resuspended in normal medium containing 250 μg/ml of kanamycin.

Indirect immunofluorescence tests were performed 48 h and 72 h after fusion. Cells were allowed to react with the rabbit anti-mouse β2-m antiserum and with fluorescein immunoconjugated goat anti-rabbit immunoglobulins, using the conventional indirect immunofluorescence procedure (Pope and Rowe, 1964). β2-m expression in transformed and parental cells was determined either by fluorescence microscopy or by flow cytofluorimetry as follows: stained cells were analyzed for the percentage of bright fluorescent cells in a cytofluorograph 50 HH (Ortho Diagnostic Systems, Westwood, MA) using the 488 nm line with 400 mW power from an Arganion laser (Lexel model 95.4). In this analysis, cells with a given size class were selected in such a way as to discriminate for living cells (dead cells have a different shape and are, therefore, not taken into account). The low angle light scattering was used for gating, thus eliminating the contribution of the debris from the fluorescence histograms. The exciting light was excluded by the dichroic filter and narrow band pass (514− 540 nm) filter for fluorescein. For each sample, data obtained from ~ 25 000 cells were analyzed on a microprocessor MP 2000 (2150 Ortho Diagnostic Systems). For each cell line, three 'windows' were selected to define three subpopulations with different mean fluorescence (see Table I).

## Acknowledgements

## References

Berg,P. (1981) *Science (Wash.)*, **213**, 296-302.

Buc-Caron,M.H., Condamine,H. and Jacob,F. (1978) *J. Embryol. Exp. Morphol.*, **47**, 149-160.

Croce,C.M., Linnenbach,A., Hebner,K., Parnes,J.R., Margulies,D.H. Appella,E. and Seidman,J.G. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 5754-5758.

Dubois,P., Fellous,M., Gachelin,G., Jacob,F., Kemler,R., Pressman,D. and Tanigaki,M. (1976) *Transplantation*, **22**, 467-473.

Gachelin,G. (1978) *Biochim. Biophys. Acta*, **516**, 27-60.

Gates,F.T., Coligan,J.E. and Kindt,T.J. (1981) *Proc. Natl. Acad. Sci. USA*, **77**, 7395-7399.

Gross-Bellard,M., Oudet,P. and Chambon,P. (1973) *Eur. J. Biochem.*, **36**, 32-38.

Grosveld,F.G., Hans-Henrik,M.D., Boer,E. and Flavell,R.A. (1981) *Gene*,

**13**, 227-237.

Hanahan,D. and Meselson,M. (1980) *Gene,* **10**, 63-67.

Hohn,B. (1980) *Methods Enzymol.,* **68**, 299-308.

Hohn,B. and Collins,J. (1980) *Gene,* **11**, 291-298.

Hyman,R. and Stallings,V. (1976) *Immunogenetics,* **3**, 75-84.

Jakob,H., Boon,T., Gaillard,J., Nicolas,J.F. and Jacob,F. (1973) *Ann. Microbiol. Inst. Pasteur,* **124B**, 269-282.

Lalanne,J.L., Delarbre,C., Gachelin,G. and Kourilsky,P. (1983) *Nucleic Acids, Res.,* **11**, 1567-1577.

Lingappa,V.R., Cunningham,B.A., Jazwinski,S.M., Hopp,T.P., Blobel,G. and Edelman,G.M. (1979) *Proc. Natl. Acad. Sci. USA,* **76**, 3651-3655.

Maxam,A. and Gilbert,W. (1980) *Methods Enzymol.,* **65**, 499-560.

Morello,D. (1978) *Transplantation,* **26**, 119-125.

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) *Cold Spring Harbor Symp. Quant. Biol.,* **10**, in press.

Murray,N.E., Brammar,W.J., Murray,K. (1977) *Mol. Gen. Genet.,* **150**, 53-61.

Natori,T., Katagiri,M., Tanigaki,N. and Pressman,D. (1974), *Transplantation,* **18**, 550-557.

Parnes,J.R. and Seidman,J.G. (1982) *Cell,* **29**, 661-669.

Pope,J.M. and Rowe,W.P. (1964) *J. Exp. Med.,* **120**, 121-125.

Rassoulzadegan,M., Binetray,B. and Cuzin,F. (1982) *Nature,* **295**, 257-259.

Rigby,P.W.J., Dieckmann,M., Rhodes,C. and Berg,P. (1977) *J. Mol. Biol.,* **113**, 237-251.

Sawicki,J.A., Magnuson,T. and Epstein,C.J. (1981) *Nature,* **294**, 450-451.

Southern,E.M. (1975) *J. Mol. Biol.,* **98**, 503-517.

Suggs,S.V., Wallace,R.B., Hirose,T., Kawashima,E.H. and Itakura,K. (1981) *Proc. Natl. Acad. Sci. USA,* **78**, 6613-6617.

Wieslander,L. (1979) *Anal. Biochem.,* **98**, 305-309.