*Proc. Natl. Acad. Sci. USA*
Vol. 79, pp. 1930–1934, March 1982
Genetics

# Assignment of the gene for $\beta_2$-microglobulin (*B2m*) to mouse chromosome 2

**(somatic cell hybrids/two-dimensional gel electrophoresis/comparative mapping/mouse Robertsonian translocation chromosomes/major histocompatibility complex)**

DAVID R. COX*, JANET A. SAWICKI*, DELLA YEE*, ETTORE APPELLA†, AND CHARLES J. EPSTEIN*‡

Departments of *Pediatrics and of ‡Biochemistry and Biophysics, University of California, San Francisco, California 94143; and †Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

*Communicated by Marian E. Koshland, November 30, 1981*

**ABSTRACT** **We have assigned the gene (*B2m*) coding for murine $\beta_2$-microglobulin (B2M) to mouse chromosome 2 by using a novel panel of Chinese hamster–mouse somatic cell hybrid clones. Because the 35 independent primary hybrids used in this study were derived from two types of feral mice, each with a different combination of Robertsonian translocation chromosomes, as well as from mice with a normal complement of acrocentric chromosomes, analysis of 16 selected mouse enzyme markers provided data on the segregation of all 20 mouse chromosomes in these hybrids. Mouse B2M was identified in cell hybrids by immunoprecipitation with a species-specific anti-mouse B2M antiserum followed by two-dimensional polyacrylamide gel electrophoresis of the immunoprecipitated polypeptides. Enzyme analysis of the segregant clones excluded all chromosomes for *B2m* assignment except mouse chromosome 2, and karyotype analysis of nine informative hybrid clones confirmed the assignment of *B2m* to this chromosome. These results demonstrate that, in the mouse, as in man, *B2m* is not linked to the major histocompatibility or immunoglobulin loci.**

$\beta_2$-Microglobulin (B2M), a nonglycosylated water-soluble protein of $M_r$ 12,000, has a highly conserved structure and extensive homology with immunoglobulin constant-region domains (1). It is found on the surface of most nucleated mammalian cells as a component of the major histocompatibility antigens but does not itself associate with the cell membrane (2–5). The precise function of B2M is unknown but it is thought to play an important role in those immunological phenomena, such as immune responsiveness, immune suppression, T–B cell interaction, and restriction of T-cell specificity, that depend on the expression of histocompatibility antigens (6).

Although genetic analysis of B2M has been hampered by its high degree of conservation, mouse–human somatic cell hybrids have been used to map the human *B2m* locus to chromosome 15 (7, 8). Despite the structural relationship of B2M to immunoglobulins and its physical relationship to histocompatibility antigens, the *B2m* locus is not linked to either the immunoglobulin or the major histocompatibility loci in man (9, 10). The chromosomal location of the gene coding for B2M in other species is not known. Recently, allelic forms of B2M in the mouse have been described, and preliminary studies have suggested linkage between *B2m* and genes coding for the Ly-4 and H-3 antigens, which have been assigned to mouse chromosome 2 (11, 6, 12).

In this paper, we report the use of a unique panel of Chinese hamster–mouse somatic cell hybrids segregating mouse chromosomes to assign the gene for B2M to mouse chromosome 2.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

## MATERIALS AND METHODS

**Animals and Cells.** An established Chinese hamster cell line (380-6) derived from V79 and deficient for hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) was obtained from Uta Francke (Yale University). These cells die if grown in hypoxanthine/aminopterin/thymidine selective medium (13). Mouse spleen cells used for fusion were derived from feral female mice generously provided by Alfred Gropp (Lübeck, Federal Republic of Germany). These mice, obtained from wild *Mus musculus* populations in Central and Northern Italy, near the cities of Abruzzi and Cremona, are homozygous for 9 metacentric chromosomes derived by centric (Robertsonian) fusion of 18 acrocentric autosomes (14). The Abruzzi (CD) mice have metacentric chromosomes in acrocentric combinations 1/7, 2/18, 3/8, 4/15, 5/17, 6/13, 9/16, 10/11, and 12/14, which are different from the acrocentric combinations found in the Cremona mice: 1/6, 2/8, 3/4, 5/15, 7/18, 9/14, 10/12, 11/13, and 16/17. Chromosome 19 and the sex chromosomes remain acrocentric in both types of mice.

**Formation of Somatic Cell Hybrids.** Hamster–mouse hybrid clones segregating mouse chromosomes were constructed as described (15). Fourteen independent primary clones were obtained from a polyethylene glycol-mediated fusion between 380-6 and Abruzzi mouse spleen cells (Abruzzi clones); 11 independent primary hybrids were obtained from a second fusion between 380-6 and Cremona spleen cells (Cremona clones). Ten additional primary hybrid clones, derived from a fusion between 380-6 and ICR mouse peritoneal macrophages containing only acrocentric mouse chromosomes, have been described (15).

**Enzyme Electrophoresis.** Cell extracts were prepared from either freshly trypsinized cells or frozen cell pellets as described (15). The following isozymes were analyzed as markers for mouse chromosomes: peptidase-3 (PEP-3, EC 3.4.11.-) for chromosome 1; adenylate kinase-1 (AK-1, EC 2.7.4.3) for chromosome 2; 6-phosphogluconate dehydrogenase (PGD, EC 1.1.1.44) for chromosome 4; β-glucuronidase (βGUS, EC 3.2.1.31) for chromosome 5; triosephosphate isomerase-1 (TPI-1, EC 5.3.1.1) for chromosome 6; glucosephosphate isomerase-1 (GPI-1, EC 5.3.1.9) for chromosome 7; glutathione reductase-1 (GR-1, EC 1.6.4.2) for chromosome 8; malic enzyme (MOD-1, EC 1.1.1.40) for chromosome 9; peptidase-2 (PEP-2, EC 3.4.11.-) for chromosome 10; acid phosphatase-1 (ACP-1, EC 3.1.3.2) for chromosome 12; nucleoside phosphorylase-1 (NP-1, EC 2.4.2.1) for chromosome 14; superoxide dismutase-1 (SOD-1, EC 1.15.1.1) for chromosome 16; glyoxylase-1 (GLO-1, EC 4.4.1.5) for chromosome 17; peptidase-1 (PEP-1; EC

Abbreviations: B2M, $\beta_2$-microglobulin; *B2m*, B2M gene.

3.4.11.-) for chromosome 18; and glutamate oxalacetate transaminase-1 (GOT-1, EC 2.6.1.1) for chromosome 19. With the exception of SOD-1 and GOT-1, mouse and Chinese hamster enzymes were distinguished by Cellogel electrophoresis and specific enzyme staining as described (16–19). Mouse and hamster SOD-1 and GOT-1 were distinguished by isoelectric focusing in polyacrylamide slab gels followed by specific enzyme staining (15, 20).

**Radiolabeling of Cells.** Cells were seeded in microtest tissue culture wells (Falcon Plastics no. 3040; 6.5-mm-diameter wells) at a concentration of $5 \times 10^4$ cells per well in either Dulbecco's modified Eagle's medium containing 10% fetal calf serum or in hypoxanthine/aminopterin/thymidine selective medium and incubated at 37°C for 18 hr. The medium in each well was then replaced with 150 μl of "labeling" medium (Dulbecco's or selective lacking methionine but with 5% fetal calf serum and 1% glutamine) to which [$^{35}$S]methionine (Amersham/Searle, ≈100 Ci/mmol; 1 Ci = $3.7 \times 10^{10}$ becquerels) was added (400 μCi/ml), and the cells were labeled at 37°C for 24 hr. Radioactive extracts were prepared by removing the radioactive medium from each well, washing each well three times in cold phosphate-buffered saline, and then adding 30 μl of cold 0.5% Nonidet P-40 (Particle Data, Elmhurst, IL) in Tris-buffered saline (150 mM NaCl/50 mM Tris/0.02% $NaN_3$/pH 7.0) to each well. Each extract was centrifuged at 10,000 × *g* for 5 min in a Beckman Microfuge B to remove unlysed nuclei, and the supernatants were stored at −80°C.

**Mouse B2M and Rabbit Anti-Mouse B2M Antiserum.** The purification of B2M from mouse liver and the preparation of rabbit anti-mouse B2M antiserum will be described elsewhere.

**Immunoprecipitation.** Radiolabeled cell proteins were immunoprecipitated according to the procedure described by Jones (21). Briefly, 10 μl of rabbit anti-mouse B2M antiserum was added to 60 μl of Nonidet P-40 extract in a Microfuge tube (39 × 10 mm). After a 15-min incubation at 4°C, 100 μl of a 10% (wt/vol) suspension of heat-killed, formalin-fixed *Staphylococcus aureus*, Cowan I strain (IgGSORB, The Enzyme Center, Boston), was added, and the mixture was incubated at 4°C for an additional 10 min. The IgGSORB-bound antigen–antibody complexes were diluted with SaC buffer (phosphate-buffered saline containing 0.5% Nonidet P-40 and 2 mM methionine) and centrifuged for 5 min at 10,000 × *g* in a Beckman Microfuge B; the pellet was washed three times with SaC buffer. The bound immune complexes were then released from the IgGSORB absorbent by resuspending the pellet in 30 μl of O'Farrell's lysis buffer A (22). The suspension was centrifuged for 5 min as before, and the supernatant was carefully removed and stored at −80°C.

**Two-Dimensional Polyacrylamide Gel Electrophoresis.** Two-dimensional gel electrophoresis of immunoprecipitates was carried out according to the method of O'Farrell (23). Nonequilibrium pH gradient gels having an approximate pH range of 4.5–7.9 were used for the first dimension, and electrophoresis in this dimension was carried out for 4 hr at 400 V. Separation in the second dimension was done on a 4.5% polyacrylamide stacking gel and a 14% polyacrylamide separation gel. Stained gels were dried under reduced pressure and exposed for 4–10 days to Kodak NS-2T no-screen x-ray film, which was then processed according to standard procedures (22).

**Chromosome Analysis.** Preparation of chromosome spreads, plating cells for mouse B2M assay, and harvesting cell pellets for isozyme analysis were generally performed within a week of each other for any given hybrid clone. Chromosomes were stained by standard trypsin/Giemsa banding methods (24).

**Statistical Analysis of Segregation Data.** Two statistical measures, κ and OR, were used to assign *B2m* to a specific mouse chromosome. κ, which is related to the φ coefficient (25), is a measure of agreement independent of sample size (26). κ will have a value of +1 when there is perfect agreement between B2M and a chromosome marker and a value of 0 when there is no agreement greater than predicted by chance. When there is less agreement than predicted by chance, κ becomes negative. The OR statistic is an inverse weighted scoring system for determining gene assignments which is independent of κ (25). The higher the OR value, the greater the association between B2M and a chromosome marker. OR was calculated as described (25). Statistical computations were performed with a computer program, MAPIT, designed to process segregation data generated from somatic cell hybrid clones. Although κ and OR are measures of agreement rather than tests of significance, previous studies have shown that agreement between κ-related statistics and the OR statistic is useful for distinguishing strong from weak data (25).

## RESULTS

**Mouse B2M Assay.** We used a rabbit anti-mouse B2M antiserum which does not crossreact with Chinese hamster B2M to assay for mouse B2M in extracts of Chinese hamster–mouse hybrid cells. [$^{35}$S]Methionine-labeled polypeptides were immunoprecipitated from an extract of mouse A9 cells by using the rabbit anti-mouse B2M antiserum or a normal rabbit serum control, and the immunoprecipitates were analyzed by two-dimensional gel electrophoresis followed by autoradiography. The anti-B2M immunoprecipitate included a prominent radioactive polypeptide (indicated by the arrow in Fig. 1*a*) which comigrated with purified mouse B2M and was not present in the normal rabbit serum immunoprecipitate (Fig. 1*b*). However, when radioactive polypeptides from an extract of Chinese hamster 380-6 cells were immunoprecipitated and analyzed as described above, the anti-B2M immunoprecipitate (Fig. 1*c*) contained no radioactive polypeptides that were not also pres-

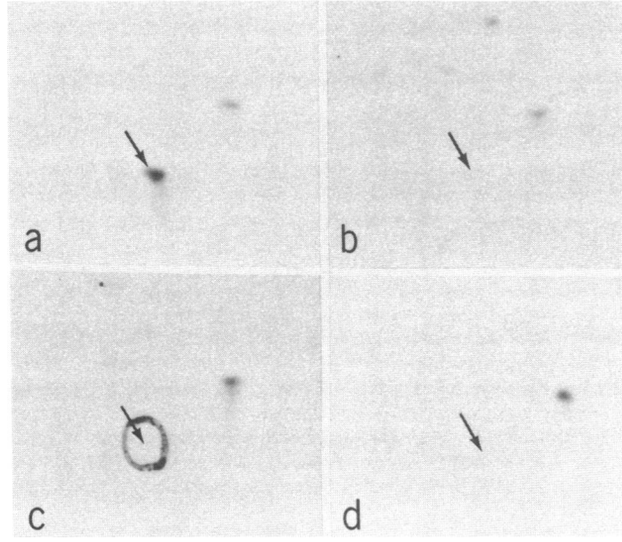


FIG. 1. [$^{35}$S]Methionine-labeled polypeptides immunoprecipitated from extracts of mouse A9 cells (*a* and *b*) or hamster 380-6 cells (*c* and *d*) by either rabbit anti-mouse B2M antiserum (*a* and *c*) or normal rabbit serum (*b* and *d*). The panels represent comparable regions from two-dimensional gel autoradiograms. Arrows: in *a*, polypeptide that comigrates with purified mouse B2M; in *b*, *c*, and *d*, comparable sites on these gels. In *c*, purified mouse B2M (2.75 μg) was added to the immunoprecipitate prior to electrophoresis. Proteins were fixed and stained with 50% trichloroacetic acid/0.1% Coomassie blue; the gel was then dried, and the stained mouse B2M spot was circled with radioactive ink.

ent in the normal rabbit serum immunoprecipitate (Fig. 1*d*). Similar results were obtained with an extract of Chinese hamster splenic lymphocytes (data not shown). Because none of the Chinese hamster polypeptides that were nonspecifically precipitated by the anti-mouse B2M antiserum and by normal rabbit serum comigrated with purified mouse B2M (Fig. 1*c* and *d*), this assay procedure can be used to identify mouse B2M unambiguously in extracts of Chinese hamster–mouse hybrid cells. A two-dimensional rather than a one-dimensional electrophoretic analysis of the immunoprecipitates is required because some of the minor, nonspecifically precipitated Chinese hamster polypeptides have molecular weights similar to the molecular weight of mouse B2M and might be mistaken for mouse B2M if the polypeptides were separated only on the basis of size.

When a series of Chinese hamster–mouse somatic cell hybrids was radiolabeled and immunoprecipitated with anti-mouse B2M antiserum and the immunoprecipitates were analyzed by two-dimensional gel electrophoresis, a radioactive polypeptide corresponding to mouse B2M was present in the immunoprecipitates from some of the clones, indicating that the gene for mouse B2M was segregating in these hybrids. Fig. 2 illustrates representative autoradiograms from hybrid clones scored as positive and negative for mouse B2M.

**Assignment of Murine *B2m* to Mouse Chromosome 2.** The segregation pattern of mouse B2M with selected mouse enzyme markers was analyzed in 14 primary hamster–mouse hybrid clones of Abruzzi origin. Because of the makeup of the Robertsonian translocations in Abruzzi mice, the mouse enzyme markers selected for analysis provided information on the segregation of all 20 mouse chromosomes in these hybrid clones. Mouse B2M segregated concordantly with the enzyme markers for the 2/18 metacentric chromosome in 10 of 11 clones and with the enzyme marker for the 3/8 metacentric chromosome in 12 of 14 clones but showed significant discordant segregation with the enzyme markers for all the other mouse chromosomes (Table 1). These results suggested that mouse *B2m* maps to chromosome 2, 3, 8, or 18.

To distinguish among these four possibilities, we analyzed the segregation of mouse B2M with mouse enzyme markers in 11 primary hamster–mouse hybrids of Cremona origin. Each of the metacentric chromosomes in Cremona mice involves a combination of acrocentric chromosomes that is different from that in Abruzzi mice. The enzyme markers analyzed in the Cremona hybrids provided information on the segregation of six of the eight metacentrics in these clones. Mouse B2M was completely concordant with the markers for the 2/8 metacentric chromosome but segregated discordantly from the markers for the 3/4 and the 7/18 metacentric chromosomes in these clones (Table 2). These results, combined with those from Table 1, indicate that mouse *B2m* maps to either chromosome 2 or chromosome 8.

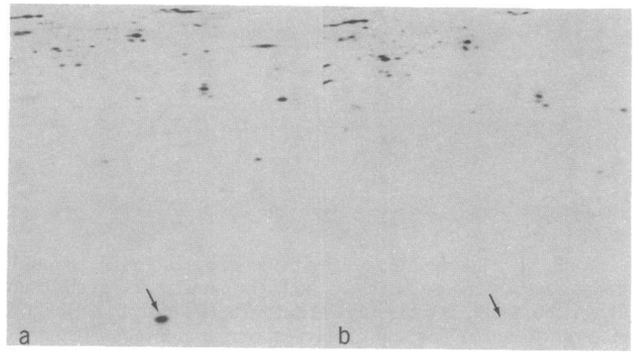


FIG. 2. Two-dimensional gel autoradiograms of [$^{35}$S]methionine-labeled polypeptides immunoprecipitated from hamster–mouse hybrid clones by using rabbit anti-mouse B2M antiserum. (*a*) Clone scored "positive" because it synthesized mouse B2M (arrow). (*b*) Clone scored "negative" (arrow points to site where mouse B2M should be). In both panels, the basic side of the gel is on the right.

Table 1. Segregation of B2M and enzyme markers in Chinese hamster–mouse somatic cell hybrids derived from Abruzzi mice

| Enzyme* | Chromosome | B2M/metacentric† +/+ | +/− | −/+ | −/− | κ | OR statistic |
|---|---|---|---|---|---|---|---|
| PEP-3/GPI-1 | 1/7 | 5 | 0 | 2 | 3 | 0.6000 | 11.36 |
| AK-1/PEP-1 | 2/18 | 8 | 0 | 1 | 2 | 0.7442 | 13.97 |
| /GR-1 | 3/8 | 8 | 1 | 1 | 4 | 0.6889 | 13.70 |
| PGD/ | 4/15 | 7 | 2 | 2 | 3 | 0.3778 | 10.30 |
| /GLO-1 | 5/17 | 7 | 2 | 2 | 3 | 0.3778 | 10.52 |
| TPI-1/ | 6/13 | 6 | 3 | 3 | 2 | 0.0667 | 7.17 |
| MOD-1/SOD-1 | 9/16 | 4 | 5 | 1 | 3 | 0.1522 | 6.76 |
| PEP-2/ | 10/11 | 2 | 7 | 0 | 5 | 0.1695 | 8.56 |
| ACP-1/NP-1 | 12/14 | 6 | 1 | 2 | 2 | 0.3774 | 10.29 |
| GOT-1 | 19 | 1 | 3 | 1 | 3 | 0.0000 | 6.72 |
| HPRT‡ | X | 9 | 0 | 5 | 0 | 0.0000 | 9.31 |

* The enzyme designations before and after the slash correspond, respectively, to the two chromosome arms of the metacentric chromosome listed in the next column.

† Number of clones. A clone was scored as positive (+) or negative (−) for a particular metacentric chromosome only when the enzyme markers for that chromosome did not indicate chromosome breakage. If a clone was positive for only one of two enzymes marking a particular metacentric chromosome, indicating chromosome breakage, data from the clone were excluded from the segregation analysis of that particular metacentric chromosome.

‡ Hamster HPRT$^-$–mouse hybrids require HPRT, assigned to the mouse X chromosome, for growth in hypoxanthine/aminopterin/thymidine selective medium.

To distinguish between these two possibilities, we combined the mouse enzyme and B2M segregation data from the Abruzzi and Cremona clones with results from an additional 10 primary hamster–mouse hybrids derived from a mouse that contained only the normal acrocentric chromosomes and no metacentric chromosomes. These combined data, representing a total of 35 primary hybrid clones, are presented in Table 3. In combining the data in this way we have not included information on those metacentric arms for which there are no direct enzyme markers. In other words, the analysis is a straightforward comparison of the segregation of mouse B2M with mouse enzyme markers, providing information on the segregation of 16 of the 20 mouse chromosomes (all but 3, 11, 13, and 15). B2M segregated concordantly with AK-1, a chromosome 2 marker, in 33 of 35 clones, but showed significantly discordant segregation with all other enzyme markers. In particular, B2M segregated discordantly

Table 2. Segregation of B2M and enzyme markers in Chinese hamster–mouse somatic cell hybrids derived from Cremona mice

| Enzyme | Chromosome | B2M/metacentric +/+ | +/− | −/+ | −/− | κ | OR statistic |
|---|---|---|---|---|---|---|---|
| PEP-3/TPI | 1/6 | 5 | 0 | 3 | 0 | 0.0000 | 10.98 |
| AK-1/GR-1 | 2/8 | 5 | 0 | 0 | 3 | 1.0000 | 30.10 |
| /PGD | 3/4 | 4 | 3 | 3 | 1 | −0.1786 | 7.71 |
| GPI-1/PEP-1 | 7/18 | 4 | 2 | 1 | 2 | 0.3077 | 17.17 |
| MOD-1/NP-1 | 9/14 | 2 | 4 | 0 | 3 | 0.2500 | 13.91 |
| PEP-2/ACP-1 | 10/12 | 4 | 2 | 1 | 2 | 0.3077 | 14.94 |
| SOD-1/GLO-1 | 16/17 | 4 | 3 | 3 | 1 | −0.1786 | 7.41 |
| HPRT | X | 7 | 0 | 4 | 0 | 0.0000 | 14.71 |

See footnotes in Table 1.

Table 3. Segregation of B2M and enzyme markers in Chinese hamster–mouse somatic cell hybrids derived from ICR, Abruzzi, and Cremona mice

| Enzyme | Chromosome | B2M/enzyme marker* +/+ | +/− | −/+ | −/− | κ | OR statistic |
|---|---|---|---|---|---|---|---|
| PEP-3 | 1 | 17 | 5 | 7 | 6 | 0.2419 | 6.64 |
| AK-1 | 2 | 22 | 0 | 2 | 11 | 0.8736 | 10.69 |
| PGD | 4 | 16 | 6 | 6 | 7 | 0.2657 | 6.09 |
| βGUS | 5 | 0 | 6 | 2 | 2 | −0.4286 | 0.68 |
| TPI-1 | 6 | 16 | 6 | 7 | 6 | 0.1918 | 5.63 |
| GPI-1 | 7 | 15 | 7 | 5 | 8 | 0.2881 | 6.57 |
| GR-1 | 8 | 18 | 4 | 2 | 11 | 0.6441 | 8.90 |
| MOD-1 | 9 | 10 | 12 | 2 | 11 | 0.2598 | 5.70 |
| PEP-2 | 10 | 12 | 10 | 4 | 9 | 0.2173 | 6.14 |
| ACP-1 | 12 | 17 | 5 | 5 | 8 | 0.3881 | 7.45 |
| NP-1 | 14 | 12 | 10 | 3 | 10 | 0.2835 | 6.51 |
| SOD-1 | 16 | 6 | 4 | 3 | 5 | 0.2222 | 5.02 |
| GLO-1 | 17 | 13 | 9 | 5 | 8 | 0.1941 | 5.57 |
| PEP-1 | 18 | 17 | 5 | 5 | 8 | 0.3881 | 7.26 |
| GOT-1 | 19 | 6 | 4 | 2 | 6 | 0.3415 | 5.89 |
| HPRT† | X | 22 | 0 | 13 | 0 | 0.0000 | 6.30 |

* Shown as number of clones. This table combines data from ICR hybrid clones containing only acrocentric mouse chromosomes with data from the Abruzzi and Cremona hybrid clones which contain metacentric chromosomes. All isozyme data have been included, even in those cases in which there was evidence that a given metacentric had been broken. Each clone was simply scored for the presence (+) or absence (−) of a particular enzyme marker, although not all clones were scored for every enzyme. No indirect data pertaining to metacentric chromosome arms for which there was no enzyme marker have been included in this table.

† Hamster HPRT⁻–mouse hybrids require HPRT, assigned to the mouse X chromosome, for growth in hypoxanthine/aminopterin/thymidine selective medium.

from GR-1, a chromosome 8 marker, in 6 of 35 clones. Thus, taken together the data from Tables 1, 2, and 3 strongly suggest that we can assign mouse *B2m* to mouse chromosome 2.

To confirm this assignment, we analyzed the chromosome constitution of nine informative hybrid clones, including the two clones discordant for B2M and the chromosome 2 enzyme marker AK-1. The karyotype analyses revealed that chromosome 2 was the only mouse chromosome consistently concordant with mouse B2M in these hybrids (Table 4), confirming the assignment of *B2m* to mouse chromosome 2.

Chromosome analysis of the Abruzzi hybrid clone III-23 was particularly informative. This clone was one of the two clones in which B2M and the chromosome 2 enzyme marker AK-1 were discordant. It was positive for AK-1 and PEP-1, consistent with the presence of an intact 2/18 metacentric chromosome, but negative for mouse B2M. Karyotype analysis of this clone revealed a rearranged 2/18 metacentric chromosome, involving a translocation of chromosomal material of unknown origin to chromosome 2 at band 2D, with absence of the segment of chromosome 2 lying distal to the break point (Fig. 3). This finding explains the discordance between B2M and AK-1 in this clone and suggests that mouse *B2m* lies somewhere in the distal half of chromosome 2 whereas *Ak-1* maps to the proximal half of this chromosome.

Karyotype analysis of the other discordant clone, I-2, which was weakly positive for AK-1 but negative for B2M, revealed no evidence of a recognizable mouse chromosome 2, suggesting that clone I-2 contains a small nonrecognizable fragment of mouse chromosome 2 which includes *Ak-1* but not *B2m*.

## DISCUSSION

The Chinese hamster–mouse hybrids used in this study were derived from two types of feral mice, each containing a different combination of Robertsonian translocation chromosomes, as well as from mice with a normal complement of acrocentric chromosomes. We and others have previously demonstrated the usefulness of Robertsonian chromosomes for gene mapping (15, 27). However, the panel of hybrids constructed for the present study is particularly novel. This clone panel allows one to use mouse isozyme markers to follow the segregation of all 20 mouse chromosomes in somatic cell hybrids.

Breakage of metacentric mouse chromosomes in the Abruzzi and Cremona clones averaged 20–25%, as measured by discordance between isozymes marking opposite arms of a given metacentric chromosome. Such chromosome breakage is not unique to hybrid clones containing Robertsonian translocation chromosomes because a similar degree of breakage has been described in Chinese hamster–mouse somatic cell hybrids segregating normal acrocentric mouse chromosomes (28). Nevertheless, chromosome breakage in Chinese hamster–mouse somatic cell hybrids clearly limits the efficiency with which a gene assignment can be made by using isozyme data alone.

The segregation of mouse B2M and the selected enzyme markers in 35 independent primary hybrid clones was analyzed by using two different statistical measures of agreement, κ and OR. Both statistical measures indicated that mouse *B2m* expres-

Table 4. Segregation of B2M and mouse chromosomes in Chinese hamster–mouse somatic cell hybrids

| Clone | B2M | Mouse chromosome* 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | X |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| III-16 | + | | + | + | | + | + | + | + | + | | | + | + | + | | + | + | + | | + |
| CIV-12 | + | | + | | | | | | + | | + | | + | | | | + | + | | | + |
| I-7B | + | + | + | | | | + | | | + | + | | | | + | | + | | + | + | + |
| I-3A-3 | + | + | + | | + | | | | | + | | | | + | | + | | | + | | |
| I-2 | − | | | | | + | | + | | | + | | | + | | | | | | | + |
| III-12 | − | | | | | + | + | | | | | | + | + | + | | | + | | | + |
| III-13 | − | + | | | | + | | + | | + | | | | | | | + | + | | | + |
| III-14 | − | + | | | + | | | + | | | | | | | | + | + | | | + | + |
| III-23 | − | | +† | + | | | | | + | | | | + | | + | | | | + | | + |

* A chromosome was scored + if it was identified in more than 15% of the metaphases analyzed. Each arm of a metacentric chromosome has been included in this table as though it were a separate chromosome.

† This clone contained a rearranged 2/18 metacentric chromosome, involving a translocation of chromosomal material of unknown origin to chromosome 2 at band 2D, with deletion of the normal chromosome 2 material distal to band 2D (see Fig. 3).

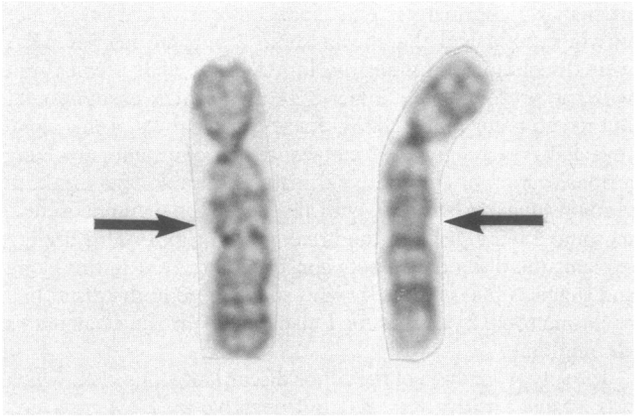

FIG. 3. Trypsin/Giemsa banding pattern of the rearranged 2/18 metacentric chromosome from clone III-23 (Left) compared with a normal 2/8 metacentric chromosome (Right). The arrows indicate the break point of the rearranged chromosome 2. Chromosomal material of unknown origin has been translocated to the proximal portion of chromosome 2 at the break point, accompanied by loss of normal chromosome 2 material distal to the break point.

sion correlated best with the presence of mouse chromosome 2. Karyotype analysis of nine informative hybrid clones confirmed the assignment of the gene for B2M to mouse chromosome 2. Our finding that a hybrid clone containing a rearranged chromosome 2, missing most of band 2E and the chromosomal material distal to it, was negative for B2M suggests that *B2m* maps to the distal half of chromosome 2.

Our results are consistent with the recent observation that the distribution of B2M isotypes in a series of recombinant inbred mouse strains follows that of Ly-4 and H-3, which are cell surface antigens encoded by genes on mouse chromosome 2 (6, 12). Although it has been suggested that B2M, Ly-4, and H-3 are identical (6), we believe that this is unlikely because Ly-4 antigen is restricted to lymphocytes whereas B2M is expressed on most nucleated cells (29). Further experiments are required to clarify the relationship between B2M and H-3.

The assignment of *B2m* to mouse chromosome 2 takes on added significance in light of the recent assignment of the mouse sorbitol dehydrogenase locus (*Sdh-1*) to this chromosome (30). Because in man *B2m* and the human sorbitol dehydrogenase locus *SORD* are syntenic, mapping to human chromosome 15 (31), the assignment of both these loci to mouse chromosome 2 provides another example of the conservation of certain autosomal linkage groups in mouse and man (15, 32). In addition, our results demonstrate that in the mouse, as in man, *B2m* is not linked to the major histocompatibility or immunoglobulin loci, despite the homology between B2M and immunoglobulin constant-region domains.

**Note Added in Proof.** After this paper was submitted, we learned that Robinson *et al.* (33) have mapped mouse *B2m* by using classical linkage analysis. Our results are in complete agreement.

We thank Dr. A. Gropp for generously providing the Abruzzi and Cremona feral mice, Mr. G. Golden for developing the MAPIT computer program, and Ms. M. J. Fredette for typing the manuscript. This work was supported by Research Grant GM-24309 and Training Grant GM-07085 from the National Institutes of Health and a Fellowship from the Johnson and Johnson Institute for Pediatric Service to D.R.C.; C.J.E. was an Investigator of the Howard Hughes Medical Institute.

1. Peterson, P. A., Cunningham, B. A., Berggard, I. & Edelman, G. M. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1697–1701.
2. Nakamuro, K., Tanigaki, N. & Pressman, D. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2863–2865.
3. Klein, J. (1979) *Science* **203,** 516–521.
4. Grey, H. M., Kubo, R. T., Colon, S. M., Poulik, M. D., Cresswell, P., Springer, T., Turner, M. & Strominger, J. L. (1973) *J. Exp. Med.* **138,** 1608–1612.
5. Dobberstein, B., Garoff, H., Warren, G. & Robinson, P. J. (1979) *Cell* **17,** 759–769.
6. Goding, J. W. & Walker, I. D. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 7395–7399.
7. Goodfellow, P. N., Jones, E. A., Van Heyningen, V., Solomon, E., Bobrow, M., Miggiano, V. & Bodmer, W. F. (1975) *Nature* **254,** 267–269.
8. Faber, H. E., Kucherlapati, R. S., Poulik, M. D., Ruddle, F. H. & Smithies, O. (1976) *Somatic Cell Genet.* **2,** 141–153.
9. Croce, C. M., Shander, M., Martinis, J., Cicurel, L., D'Ancona, G. G., Dolby, T. W. & Koprowski, H. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3416–3419.
10. Van Someren, H., Westerveld, A., Hagemeijer, A., Mees, J. R., Khan, P. M. & Zaalberg, D. B. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 962–965.
11. Michaelson, J., Rothenberg, E. & Boyse, E. A. (1980) *Immunogenetics* **11,** 93–95.
12. Robinson, P. J., Graf, L. & Sege, K. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 1167–1170.
13. Littlefield, J. W. (1964) *Science* **145,** 709–710.
14. Capanna, E., Gropp, A., Winking, H., Noack, G. & Civitelli, M. V. (1976) *Chromosoma* **58,** 341–353.
15. Cox, D. R., Epstein, L. B. & Epstein, C. J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2168–2172.
16. Meera Khan, P. (1971) *Arch. Biochem. Biophys.* **145,** 470–483.
17. Van Someren, H., Beijersbergen Van Henegouwen, H., Los, W., Wurzer-Gifurelli, E., Doppert, B., Vervloet, M. & Meera Khan, P. (1974) *Humangenetik* **25,** 189–201.
18. Wijnen, L. M. M., Grzeschik, K.-H., Pearson, P. L. & Meera Khan, P. (1977) *Hum. Genet.* **37,** 271–278.
19. Meera Khan, P. & Doppert, B. A. (1976) *Hum. Genet.* **34,** 53–56.
20. Schäfer, R., Dochmer, J., Rademacher, I. & Willecke, K. (1980) *Somatic Cell Genet.* **6,** 709–719.
21. Jones, P. P. (1977) *J. Exp. Med.* **146,** 1261–1279.
22. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250,** 4007–4021.
23. O'Farrell, P. Z., Goodman, H. M. & O'Farrell, P. H. (1977) *Cell* **12,** 1133–1142.
24. Francke, U. & Oliver, N. (1978) *Hum. Genet.* **45,** 137–165.
25. Cowmeadow, M. P. & Ruddle, F. H. (1978) *Cytogenet. Cell Genet.* **22,** 694–697.
26. Cohen, J. (1968) *Psychol. Bull.* **70,** 213–220.
27. Hengartner, H., Neo, T. & Müller, E. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4494–4498.
28. Francke, U., Lalley, P. A., Moss, W., Ivy, J. & Minna, J. D. (1977) *Cytogenet. Cell Genet.* **19,** 57–84.
29. Potter, T. A., McKenzie, I. F. C. & Cherry, M. C. (1981) *J. Immunogenet.* **8,** 15–25.
30. Peters, J. & Andrews, S. J. (1980) *Mouse News Lett.* **63,** 18.
31. Donald, L. J., Wang, H. S. & Hamerton, J. (1979) *Cytogenet. Cell Genet.* **25,** 148.
32. Lalley, P. A., Minna, J. D. & Francke, U. (1978) *Nature (London)* **274,** 160–163.
33. Robinson, P. J., Lundin, L., Sege, K., Graf, L., Wigzell, H. & Peterson, P. A. (1981) *Immunogenetics* **14,** 449–452.