prefrontal and piriform cortices (Fig. 2); unlike the more severe shock conditions[3–5], however, there was no change in DOPAC levels in the nucleus accumbens (Fig. 2). As expected, the levels of DOPAC in cingulate cortex and striatum also were unchanged after footshock[3–5] (Fig. 2).

Lisoprawski *et al.*[6] reported a marked depletion of SP in the interpeduncular nucleus and the VTA of rat brain after exposure to footshock. However, we found that the more mild footshock regime used had no effect on SP levels measured by radioimmunoassay[10] in combined interpeduncular nucleus and VTA (unshocked: 399.7 ± 18.2 pmol g^{-1} SP, $n = 7$; shocked: 378.8 ± 32.3 pmol g^{-1} SP, $n = 8$). The footshock-induced changes in DOPAC in the prefrontal and piriform cortices might nevertheless be mediated by changes in SP turnover without any change in SP levels. To test this hypothesis 10 μg per side of a rat monoclonal antibody to SP (NCl/34, Sera-Lab) was infused through stereotaxically implanted cannulae into the VTA immediately before footshock (Fig. 1). This completely prevented the increase in prefrontal and piriform cortex DOPAC normally elicited (Fig. 2). In control experiments, pre-infusion (Fig. 2) of 10 μg per side of a rat monoclonal antibody to mouse IgG (Sera-Lab) did not prevent the footshock-induced increase in prefrontal cortical DOPAC (unshocked 62.9 ± 2.9 ng g^{-1}, $n = 8$; shocked: 85.4 ± 4.4* ng g^{-1}, $n = 9$, $P^* < 0.02$)).

The combination of footshock plus pretreatment with monoclonal antibody against SP produced DOPAC levels in the nucleus accumbens and striatum significantly below those in unshocked controls (Fig. 2). This result is not understood. The lack of effect of monoclonal antibody infusion alone on DOPAC levels (Fig. 2) suggests that the SP input to the VTA may exert little tonic control of the activity of DA cells originating in the VTA. In contrast, Chéramy *et al.*[11] found that infusion of SP antiserum into the SN of anaesthetized cats decreased the activity of nigrostriatal DA cells, suggesting that in the cat the SP input to the SN does exert a tonic excitatory influence on those DA cells.

The DA cells with fibres innervating the prefrontal cortex lack autoreceptors, while those DA cells projecting to the piriform cortex possess autoreceptors[12–15]. Yet both these mesocortical DA systems are activated by footshock via a SP-dependent mechanism. This suggests that DA autoreceptors are not involved in response to footshock.

The prevention of footshock-induced changes in prefrontal and piriform cortex DOPAC by prior infusion of monoclonal antibody against SP into the VTA supports the notion that release of SP in the VTA is involved in the activation of mesocortical DA neurones in response to footshock stress.

We thank L. Bird for assistance in the preparation of Fig. 1, and S. West and M. Wynn for help with the preparation of this manuscript. M.J.B. is a John R. Whittier Fellow of the Committee to Combat Huntington's Disease, P.J.E. is a MRC Student, J.E.A. is a Marshall Scholar and M.G. is a British Council Scholar.

Received 25 April; accepted 13 October 1983.

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A potential donor gene for the *bm1* gene conversion event in the C57BL mouse

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The mammalian major histocompatibility complex (MHC; *H-2* complex in mouse) is a large multigene complex which encodes cell-surface antigens involved in the cellular immune response to foreign antigens[1]. Class I polypeptides expressed at the *H-2K* and *H-2D* (Fig. 1) loci of numerous mouse strains exhibit an unusually high degree of genetic polymorphism, which is assumed to be related to their function as primary recognition elements in the immune response. We suggested that this *H-2* polymorphism may arise by gene conversion-like events between non-allelic class I genes. This is supported by our recent comparison of the DNA sequences of the normal $H\text{-}2K^b$ gene sequence, from the C57BL/10 mouse, and a mutant[2,3] form of this gene called $H\text{-}2K^{bm1}$ (ref. 4): the mutant allele differs from the $H\text{-}2K^b$ gene in seven bases out of a region of 13 bases in exon 3 of the class I gene (which encodes α_2 (C1) the second highly polymorphic protein domain), suggesting that this region of new sequence had been introduced into the $H\text{-}2K^b$ sequence following unequal pairing of two class I genes in the genome of the C57BL mouse. Schulze *et al.* have obtained similar results[5]. Here we report work identifying a potential donor gene in our library of 26 class I genes cloned from the C57BL/10 mouse.

The region of seven clustered base changes between the $H\text{-}2K^b$ and the $H\text{-}2K^{bm1}$ genes is shown in Fig. 2*A*. As a probe for other class I genes which possess the *bm1*-specific sequence, we synthesized a 15-base oligonucleotide complementary to the *bm1*-specific sequence (Fig. 2*A*) and labelled it at the 5′ end using [γ^{32}P]ATP and polynucleotide kinase. The radiolabelled oligonucleotide was then hybridized, in optimal conditions, to *Bam*HI digests of a selection of cosmids containing *H-2* class I genes isolated from a C57BL/10 spleen library (L. Golden, manuscript in preparation). These cosmids contain a total of 26 different class I gene-related sequences as shown by restriction enzyme and hybridization analysis of the cosmid inserts with 5′ and 3′ class I gene probes. We believe that these genes are most, if not all, of the class I genes in the C57BL/10 genome. The result of the oligonucleotide hybridization to these cosmids is shown in Fig. 2*B*.

The *bm1* specific oligonucleotide hybridizes strongly to a class I gene in cosmid B1.30 (1.3 kilobase (kb) *Bam* fragment) and to no other cloned class 1 genes from C57BL/10 mouse DNA. Cosmid BM1–11 is included as a positive control because it was isolated from a *bm1* cosmid library and contains the $H\text{-}2K^{bm1}$ gene[4]. In the conditions used, the $H\text{-}2D^b$ gene (cosmid B4.15) does not hybridize to the oligonucleotide probe (data not shown) despite the fact that the DNA sequence matches at 14 out of 15 positions (see ref. 6). Nevertheless, we have seen unreproducible, weak hybridization of the oligonucleotide probe to large *Bam*HI fragments from other cosmids which in some cases (for example, *H26* in Fig. 2*B*) do not even hybridize to class I gene probes. In one case (cosmid B3.21, not shown), we sequenced through the exon 3 region of the class I gene on such a *Bam*HI fragment and found a sequence with a single mismatch to the *bm1* oligonucleotide probe at amino acid positions 152–156. In contrast, the *bm1* oligonucleotide hybridizes very strongly to the 1.3 kb *Bam*HI fragment in cosmid B1.30 suggesting that there is a perfect match to the *bm1* oligonucleotide probe in

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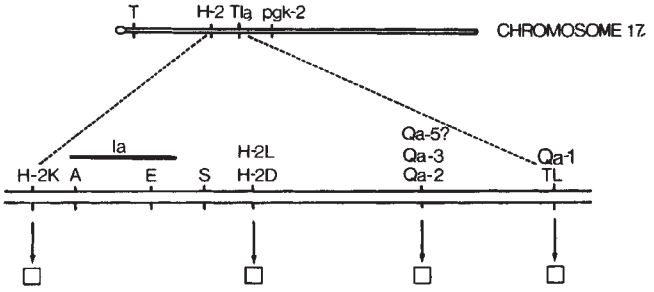

Fig. 1 Genetic map of *H-2* and associated genetic loci on chromosome 17 (top line). Four genetic loci within the *H-2* and *Tla* regions(□) control the expression of class I polypeptides (bottom line).

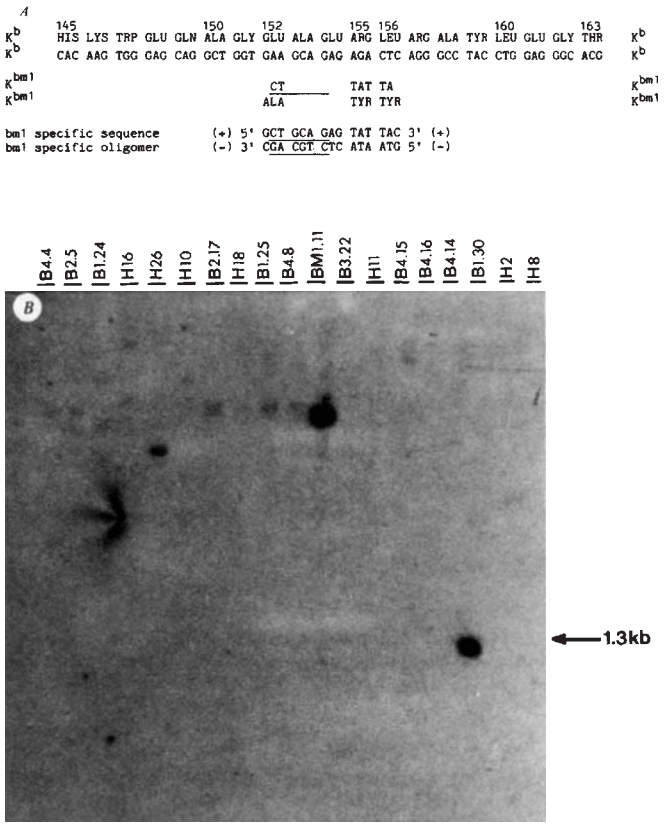

Fig. 2 *A*, Comparison of DNA and amino acid sequences of the *H-2K*^{b} (K^b) and the *H-2K*^{bm1} (K^{bm1}) genes from amino acid 145 to 163. The 15-base *bm1*-specific sequence (+) is shown below the K^{bm1} sequence. The complementary 15-base sequence (−) on the bottom line was synthesized manually on a silica gel polymer support by a modification of the published procedure[12]. The 5′-OH generated by this procedure was phosphorylated in the presence of [γ^{32}-P]ATP (Amersham) and T4 polynucleotide kinase (NE Biolabs)[7]. *Pst*I sites in the *bm1*-specific sequences are underlined. *B*, Hybridization of the 15-base radiolabelled oligonucleotide probe in *A* to *Bam*HI digests of cosmids containing class I genes. 0.6 μg of each cosmid clone was digested with *Bam*HI, run on a 0.6% agarose gel and transferred to nitrocellulose by the Southern blotting procedure[13]. The filters were prehybridized for 2 h in 6×NET (1×NET, 0.15 M NaCl, 0.03 M Tris HCl, *p*H 8.0, 1 mM EDTA), 5×Denhardts, 0.5% Nonidet P-40, 20 μg ml^{-1} denatured *coli* DNA at 65 °C. Hybridization was performed overnight at 34°–36 °C in 6×NET, 5×Denhardts, 0.5% Nonidet P-40 and 6×10^6 c.p.m. of ^{32}P-labelled *bm1*-15mer. The filters were washed twice in 3×SSC, 0.1 SDS, twice in 1×SSC, 0.1 SDS, twice in 0.1×SSC for 20 min at hybridization or room temperature[14].

this class I gene. Cosmid B1.30 contains one complete class I gene and is a member of a large overlapping family of cosmids which together define a region of ~200 kb containing 10 class I genes. The gene (*Q*10) in cosmid B1.30 maps to one end of this region of DNA which has been shown to map to the *Qa*2.3 locus by polymorphic restriction enzyme analysis. Further details of this region of clustered class I genes will be published elsewhere (L. Golden *et al.*; manuscript in preparation).

The 1.3-kb *Bam* fragment hybridizing to the *bm1*-specific oligonucleotide in Fig. 2*B* was subcloned into the vector pBR327 and restriction maps were deduced as a prelude to DNA sequence analysis. We were able to focus on the region hybridizing to the *bm1*-specific oligonucleotide probe since the 15 bp region includes a *Pst*I site (Fig. 2*A*). The 1.3-kb *Bam* fragment contains a ~200-bp *Bam*HI–*Pst*I fragment at one end of the subclone which hybridized strongly to 5′ class I gene probes containing exon 3. DNA sequence analysis of this region was carried out using the modified Maxam and Gilbert chemical sequencing procedure[7] (Fig. 3). The DNA sequence obtained is homologous to the DNA sequences found in exon 3 (encodes the α_2 protein domain) of all class I genes which have been sequenced to date[6,8–10]. In Fig. 4 we present the entire DNA sequence of exon 3 of the *H-2K*^{b} (see ref. 8) gene and show underneath a comparison of the corresponding homologous sequence obtained from the 1.3-kb *Bam*HI subclone of the *Q*10 gene. The DNA sequence which encodes amino acids 152–156 is identical to the sequence of the *bm1*-specific oligonucleotide probe. This confirms that the *Q*10 gene is a potential donor gene for the *H-2K*^{b} to *H-2K*^{bm1} gene conversion event. In addition, the sequences of this exon 3 region have apparently normal splice acceptor and donor sequences and, moreover, have open reading frames which correlate exactly in size to the *H-2K*^{b} exon 3 sequence. It is possible, from the DNA sequence, to predict the maximum extent of the sequence transfer if the *Q*10 gene did take part in the *H-2K*^{b} to *H-2K*^{bm1} conversion event. Since the DNA sequences of the *Q*10 and the *H-2K*^{b} genes first diverges 21 bases to the left of the *bm1*-specific sequence (at amino acid residue 145) and 17 bases to the right (at amino acid residue 162), the maximum extent of gene conversion between these genes is 51 bases.

Since the *bm1* mutation was first detected in a (C57BL/6× BALB/c)F_1 mouse[2] it is also possible that the *H-2L*^{d} gene was the donor gene for this event. In this case, the actual transfer of DNA sequence information from the *H-2L*^{d} to the *H-2K*^{b} gene would have had to take place as in interchromosomal event in the zygote as we have stated previously[4]. The maximum extent of gene conversion which could have occurred if the *H-2L*^{d} gene (bottom line, Fig. 4) had been the donor gene for the *bm1* conversion is 53 nucleotides. It might be argued that the sequence of the *Q*10 gene in the C57BL/6 mouse differs from that of the C57BL/10 subline. We believe that sequence differences are unlikely to exist for two reasons. First, we have detected only eight base changes (out of a total of ~2,500 nucleotides compared) between the *H-2K*^{b} (C57BL/10) and *H-2K*^{bm1} (C57BL/6) genes[8] and seven of these changes are due to the *bm1* mutation itself. This is strikingly high conservation of two *H-2K* genes in a region of otherwise high DNA polymorphism between different mouse strains. Second, we have found, from further sequence analysis of the *Q*10 gene, that the *Q*10 gene sequence is almost identical (99.4% conserved) with the sequence of a class I cDNA from a different (SWR/J, *H-2*^{q} haplotype) mouse[16], suggesting that the cDNA sequence is allelic to the *Q*10 gene. Thus, if the gene sequence is conserved between two different inbred strains it seems unlikely that there will be any significant variation between the two C57BL sublines.

The discovery that a donor gene for the *bm1* conversion event exists in the C57BL genome provides extra support to our

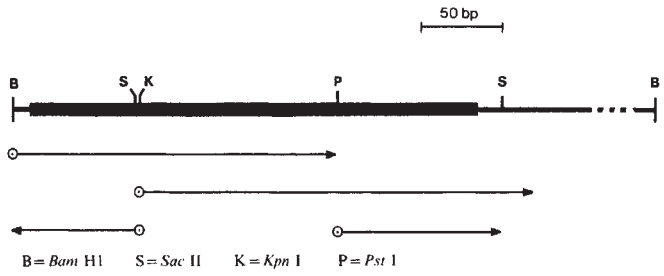

Fig. 3 Strategy for DNA sequencing of exon 3 of the *Q*10 gene. Partial restriction map of one end of the 1.3-kb *Bam*HI fragment from cosmid B1.30 which hybridizes strongly to 5′ gene probes. Fragments for Maxam and Gilbert sequencing were prepared by labelling the *Bam*HI (B), *Kpn*I (K) or *Pst*I (P) sites (⊙) with T4 polynucleotide kinase[7] or 3′ terminal labelling kit (supplied by Amersham; cordycepin/terminal transferase; see ref. 15), respectively. After labelling, fragments were redigested with *Pst*I, *Bam*HI or *Sac*II, respectively, isolated from low melting agarose gels and subjected to modified Maxam and Gilbert base-specific chemical degradation reactions[7]. The degraded fragments were then separated on a series of 6% acrylamide gels. The thick line represents the extent of exon 3 in the sequence (see Fig. 4) obtained by this procedure. Arrows indicate the extent of DNA sequence information obtained from each labelled fragment.

previous proposal that gene conversion events between non-alleles have an important role in the generation of *H-2* polymorphism at the *H-2K* and *H-2D* loci. Even so, the exact molecular mechanism whereby sequences are transferred between homologous, non-allelic genes is obscure. If unequal pairing of homologous genes is a prerequisite for inter-gene sequence transfer, then the subsequent molecular mechanism of transfer may involve either reciprocal-strand exchange between genes or replication-dependent correction of one gene sequence using a template derived from the donor gene. The data do not distinguish between these two mechanisms. One objection to the reciprocal recombination model, however, arises from our recent findings that the *Q*10 gene sequence is highly conserved amongst different mouse strains, suggesting that reciprocal mutation events involving the *Q*10 gene do not take place. Whatever the precise molecular mechanism, the data presented here suggest that very small regions of DNA are transferred from one gene to another by this mechanism (13–51 bases for the *bm1* mutation). This agrees with our previous conclusions about the molecular basis of *H-2* polymorphism which we obtained whilst comparing the $H\text{-}2K^b$ sequence with the allelic $H\text{-}2K^d$ and non-allelic $H\text{-}2L^d$ sequence[8]. In this latter study, we found that base differences in the allelic gene pair frequently occur in clusters, especially in exons 2 and 3 which encode the polymorphic α_1 and α_2 protein domains. Furthermore, the $H\text{-}2L^d$ sequence is often identical to either the $H\text{-}2K^b$ or the $H\text{-}2K^d$ sequence at these regions of clustered base changes, suggesting that the class I gene sequence in these exons should be regarded as mosaics of 'mini-gene' sequences which are reshuffled between non-allelic (and allelic?) class I genes by gene conversion-like events.

Since other *bm* mutations[3] have taken place in exon 3 of the $H\text{-}2K^b$ gene, we checked the sequence of the *Q*10 gene for the corresponding sequence of each *bm* mutant to see if this gene could be a donor for other mutations. In no case did a *bm* mutant sequence occur at a homologous position in the *Q*10 gene; the *Q*10 gene is not therefore, the exclusive donor gene for *H-2* mutations at the *H-2K* locus. Presumably, other class I genes in the C57BL genome contain DNA regions corresponding in position and sequence to the other *bm* mutations. We know from mapping data to be presented elsewhere (L. Golden *et al.*, manuscript in preparation), that the *Q*10 potential donor gene maps to the *Qa*2.3 locus. Thus, although sequence transfer appears to take place over short regions of DNA, the two genes involved in gene conversion can be widely separated on the chromosome: the *H-2K* and *Qa*2,3 loci are about centimorgan apart, as much as 2,000 kb DNA on the chromosome. This suggests that unequal gene pairing may take place over large chromosomal distances. It cannot be excluded, however, that gene conversion events are mediated by *trans*-acting nucleic acid sequences, such as messenger RNA.

We thank Jo-ann Jablonski for technical assistance, Richard Tizard for advice and Madlyn Nathanson for preparing the manuscript. This work was supported by Biogen NV.

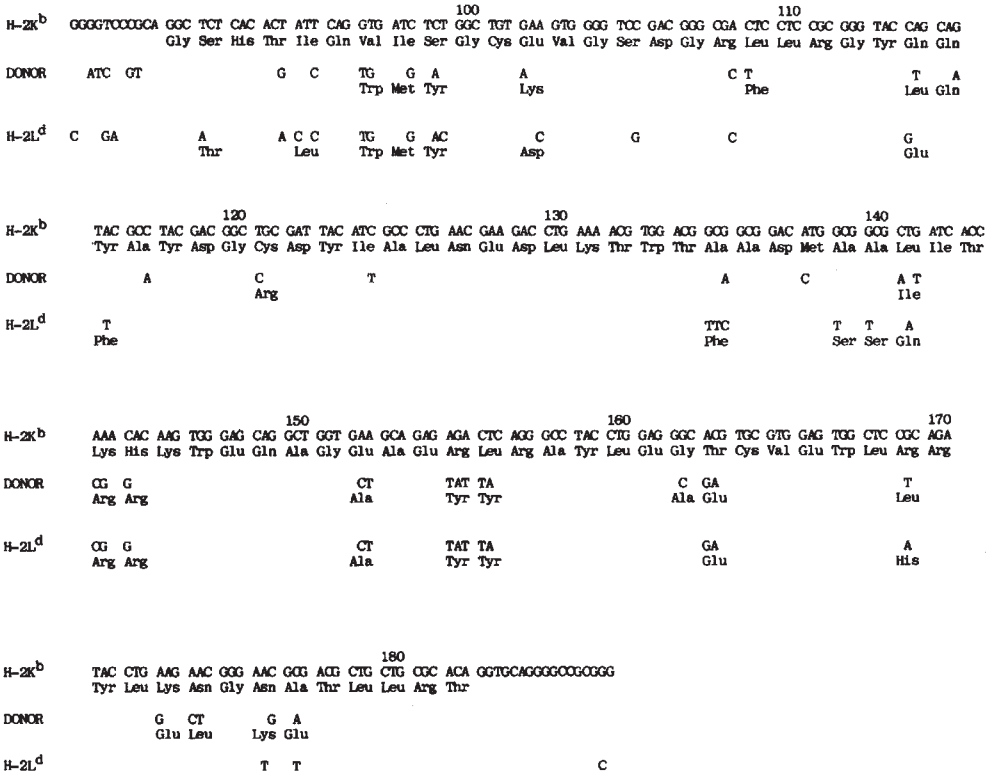

Fig. 4 Comparison of DNA and amino acid sequences of the $H\text{-}2K^b$ gene (top line; from ref. 8), the *Q*10 donor gene (centre line) and the $H\text{-}2L^d$ gene (bottom line; from ref. 9).

Received 29 July; accepted 18 October 1983.

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Rearranged c-*mos* locus in a MOPC 21 murine myeloma cell line and its persistence in hybridomas

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Studies of a number of normal and carcinogen-transformed murine cell lines, and a variety of murine tissues, have indicated that, in contrast to several other cellular oncogenes, the oncogene c-*mos* gene is usually transcriptionally silent[1,2]. The recent report by Rechavi *et al.*[3] indicating that in the mouse myeloma XRPC24 originally induced by pristane (2,6,10-14-tetramethylpentadecane) the c-*mos* gene is rearranged and transcriptionally active, and that it can transform murine fibroblasts in a transfection assay, is therefore of considerable interest. Here we show that the c-*mos* locus has undergone a similar rearrangement, and is also transcriptionally active, in the cell line P3-X63-Ag8-653, a derivative of the mouse myeloma MOPC 21 which was induced by mineral oil[4,5]. This line is widely used for making hybridomas that synthesize monoclonal antibodies[6,7]. We also demonstrate that the rearranged c-*mos* sequence is maintained in three different hybridomas derived by fusion of this cell line with normal murine spleen lymphocytes, suggesting that it may play a role in the continuous growth and/or constitutive immunoglobulin production by these hybridomas.

Myelomas can be readily induced by intraperitoneal injection of mineral oil or pristane in BALB/c mice[5]. Potter *et al.*[5] have suggested that only a few genes determine the resistance or susceptibility to myeloma induction by this procedure in various strains of mice. We have examined the arrangement of the c-*mos* sequence in the genome of the myeloma cell line P3-X63-Ag8-653 (abbreviated P3) a derivative of the MOPC 21 myeloma[4,5]. This line is of particular interest since it is used extensively for the development of hybridomas, because it does not express immunoglobulin chains and fuses very efficiently with antibody-forming cells to form immortal hybrid cell lines that produce homogeneous monoclonal antibodies[4].

DNA samples obtained from P3 cells and various other cell types were cleaved with specific restriction endonucleases, electrophoresed through a 1% agarose gel, blot hybridized by the Southern procedure[8] with a probe specific for c-*mos*[9] and radioautographs of the filters were made. Figure 1 indicates that with DNA from the murine fibroblast cell line NIH 3T3, cleavage with the restriction enzymes *Eco*RI, *Hin*dIII, *Sac*I or *Xba*I gave single bands homologous to the c-*mos* probe, whose sizes were 14.5, 8.7, 6.6 and 2.8 kilobase pairs (kb), respectively. Identical

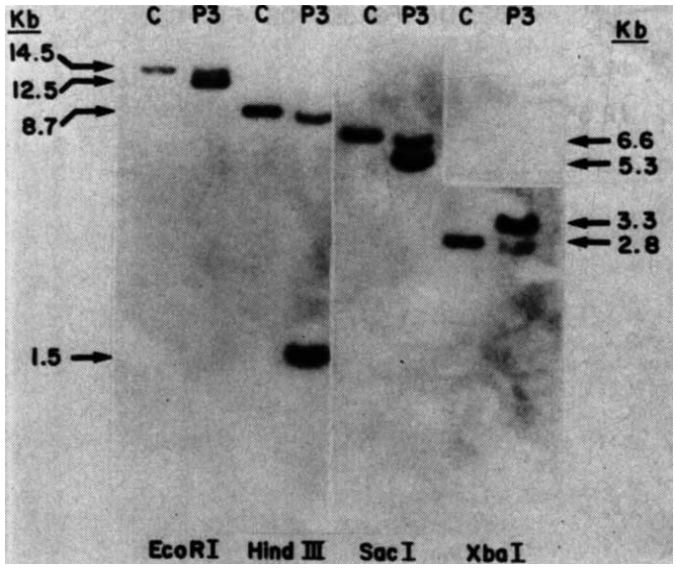

Fig. 1 Southern[8] blot patterns of the c-*mos* sequences in the genome of NIH 3T3 (C) and murine myeloma cells (P3). Chromosomal DNAs were digested with the restriction endonucleases (New England Biolabs) shown in the figure using the conditions suggested by the manufacturer. The DNAs (10 μg per lane) were electrophoresed through a 1% agarose gel, transferred onto nitrocellulose filter paper and hybridized with a ^{32}P-labelled[10] probe prepared from an *Ava*I–*Hin*dIII c-*mos* specific fragment contained in the plasmid pMS1[9], kindly provided by Dr George Vande Woude. After hybridization the blots were subjected to autoradiography. The molecular weights of the normal and rearranged c-*mos* alleles are shown by the arrows.

results were obtained with DNA samples from the BALB/c 3T3 fibroblast cell line, two carcinogen-induced transformants of this cell line and normal liver of BALB/c mice (results not shown). In a previous study[1], *Sac*I-cleaved DNA samples from a variety of murine cell types also yielded a single band of about 7 kb homologous to a v-*mos* probe. These samples included DNAs from: C3H 10T1/2 fibroblasts, a variety of carcinogen and virus-transformed murine fibroblast cell lines, an AKR (spontaneous) lymphoma, an RF murine lymphoma (methylcholanthrene induced), the F9 murine teratocarcinoma cell line, and several normal murine tissues (spleen, liver, brain, kidney). On the other hand, we now find that with the P3 DNA there appears to be rearrangement of at least one of the c-*mos* alleles, since two rather than one c-*mos* homologous bands were obtained with all four of the restriction enzymes tested. Thus, *Eco*RI digestion yielded 14.5 and 12.5 kb bands, *Hin*dIII gave 8.7 and 1.5 kb bands, *Sac*I gave 6.6 and 5.5 kb bands and *Xba*I gave 3.3 and 2.8 kb bands (Fig. 1).

We also examined three hybridoma cell lines B2, D6 and F8 which were formed by the fusion of P3 cells with normal spleen cells from immunized mice and were selected for the synthesis of monoclonal antibodies to anti Bis Q (B. Erlanger, personal communication). Figure 2 indicates that like the P3 cell line (see Fig. 1), and in contrast to the control sample, all three of the hybridomas displayed two bands with *Eco*RI-cleaved DNA (14.5 and 12.5 kb) and with *Sac*I-cleaved DNA (6.6 and 5.3 kb). As with the parental P3 cells, the additional band was in general more intense than the normal band. This was particularly striking with the F8 hybridoma, with both *Eco*RI and *Sac*I cleavage (Fig. 2). We have consistently observed this difference in intensity between the normal and additional band in the P3 and hybridoma cell lines, suggesting that the altered c-*mos* allele may have also undergone amplification.

Poly(A)$^+$RNA preparations obtained from P3 cells grown under three different conditions (see Fig. 3 legend) gave a single hybridizing band with an apparent size of about 1.2 kbp, when electrophoresed and blot-hybridized to a ^{32}P-labelled c-*mos*

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