Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 9921–9925, October 1994
Genetics

A determination of the frequency of gene conversion in unmanipulated mouse sperm

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Communicated by Phillippa Marrack, June 6, 1994

ABSTRACT **Gene conversion, sometimes also called micro gene conversion or gene conversion-like events, has been proposed to act on a number of genes in higher eukaryotes, such as γ-globin, β-tubulin, major urinary protein, and amyloid A genes. In the immune system, immunoglobulin genes and major histocompatibility complex class I and class II genes have been implicated. The notion that integral segments of DNA have been transferred from one gene to another in these cases has, however, met considerable resistance. We have devised a PCR assay detecting only the molecule that results if the Eβ^d-derived fragment analogous to that introduced in the bm12 mutation is transferred to the Aβ^k gene. We have proceeded to analyze sperm from the F_1 cross C3H/HeJ (haplotype k) × BALB/c (haplotype d). In our assay, we find that the frequency for conversion of this particular DNA segment is 2×10^{-6}. This frequency is relevant only in the germ line; when liver cells were tested as an example of somatic cells, no events were observed, implying a frequency of $<2 \times 10^{-8}$ in liver. Fragments >100 bp seem to be possible to transfer in this conversion.**

Gene conversion means a transfer of genetic information from a donor gene to an acceptor gene without the donor being changed in the process. The term was originally defined in fungi, where it implies a deviation from the expected Mendelian 2:2 ratio in tetrads of haploid spores [for a review, see Radding (1)]. In 1980, the first suggestion came of a similar phenomenon in mammals, when extensive homology was found between the two fetal γ-globin genes (2). Soon afterwards, it was proposed that gene conversion was responsible for the patchy homology patterns observed in the immunoglobulin germ-line variable region sequences (3). The transfer of the term "gene conversion" to mammalian genetics meant that the definition broadened, as it is virtually impossible to assess the fate of all four products of meiosis and, therefore, to ascertain a nonreciprocal transfer in higher eukaryotes. The definition implicit in this usage of the term is rather "templated segmental mutation." In this sense, the term has been used widely in the literature for more than a decade (see, for instance, refs. 3–18). During this time, gene conversion has been used to explain both extensive homogenization between homologous genes (4–6) and generation of polymorphism by transfer of entire polymorphic segments (7–13). A genetic region where signs of gene conversion have been reported on numerous occasions is the major histocompatibility complex (MHC) (8–13, 19). Here, it has been proposed that gene conversion is a major factor in creating polymorphism in the MHC gene. The idea of a transfer of whole DNA segments from one gene to another in MHC genes without unequal crossing over has, however, been vigorously contested by Klein (20), who argues that the patchwise homologies found in MHC class I and class II genes (and also, presumably, in immunoglobulin genes) are due to selective pressure and restrictions on variability at certain sites in the genes. In the absence of an assay to detect gene conversion *in vivo*, as it occurs before selection, the argument has not been possible to resolve. Such an assay became conceivable with the advent of the PCR amplification (21), and feasible when PCR was used to amplify single-copy genes from single sperm (22). We have therefore proceeded to construct a PCR assay that can detect a gene conversion event in one sperm out of a pool of several hundred thousand by using one primer specific for the donor gene and one specific for the acceptor gene in each amplification.

MATERIALS AND METHODS

Mice. BALB/c, C3H/HeJ, and CBA mice and (C3H/HeJ × BALB/c)F_1 and (CBA × BALB/c)F_1 hybrids were bred and kept at the animal facility of the University of Stockholm.

Isolation of DNA and PCRs. Sperm cells were isolated from the epididymis by cutting epididymes to small pieces, teasing the pieces between tweezers, and vortex mixing heavily to release the sperm. For the preparation of DNA, the sperm cells were digested in a proteinase K (1%)/SDS (200 μg/ml) buffer in the presence of 1 mM dithiothreitol before phenol/chloroform extraction and ethanol precipitation. Liver DNA was prepared from animals starved for 18 h. Sperm DNA (150,000 haploid copies) or liver DNA (800,000 haploid copies) was used in a PCR. The number of copies was calculated from A_{260} values by assuming that 1 A_{260} unit = 50 μg of DNA per ml and that the molecular weight of the haploid mouse genome is 2.15×10^{12}. For the detection of gene conversion events, primers 29 and 13 (each at 10 pmol) were used for 40 cycles. One microliter of the resulting PCR product was removed and subjected to 22 additional PCR cycles with primers 13 and 14 (each at 10 pmol). For the detection of unequal crossing-over events, primers 29 and 35 were used for 40 cycles, whereupon 1 μl of the resulting product was amplified for 22 cycles with primers 14 and 13 (see Fig. 1 for primer designations). All PCRs were performed with a cycle of 94°C for 40 sec, annealing for 1 min, and extension at 74°C for 1.5 min on a Techne PHC-2 thermal cycler. Annealing was at 66°C for amplifications with primer 13 as 3′ primer and at 62°C when primer 35 was employed. A standard PCR buffer with all four dNTPs (each at 100 μM) and 2 mM Mg^{2+} was used for all reactions. Several negative controls, containing no DNA, were included in every PCR experiment.

Construction of Positive Control Template DNA. The DNA used as a positive control to verify the efficiency of the PCR amplifications was prepared in the following way: C3H/HeJ sperm DNA was PCR-amplified with primer 29 and a control primer hybridizing to the segment of the Aβ^k gene that corresponds to the primer 13 sequence in the Eβ^d gene. BALB/c sperm DNA was amplified with primer 35 and a control primer hybridizing to the segment of the Eβ^d gene that corresponds to the primer 29 sequence in the Aβ^k gene. The

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Abbreviation: MHC, major histocompatibility complex.

fragments were purified with Geneclean II (Bio 101), and the size and purity of the products were verified on an agarose gel. We then mixed the two PCR products and digested the mixture with the restriction endonuclease *Taq* I, which cuts at a unique site in the same position in both fragments. We ligated the mixture with T4 DNA ligase, chromatographed the ligation products on a preparative agarose gel, excised the fragment of the proper size, and purified it as above. The excised band was amplified on a preparative scale with primers 29 and 35, purified as above. The concentration was determined by A_{260} measurement as above.

DNA Sequence Analysis. DNA sequencing was performed with a *Taq* DyeDeoxy terminator cycle sequencing kit from Applied Biosystems according to the manufacturer's instructions and the resulting terminated products were separated and analyzed on a model 373A automated DNA sequencer, also from Applied Biosystems.

RESULTS

Setting Up a PCR Assay for Gene Conversion Events. There are several implications that have to be taken into account when one sets up a PCR assay for gene conversion. The conversion event must produce a change of sequence. Therefore, only gene conversion between polymorphic genes can be measured. MHC genes are the only polymorphic genes where individual gene conversion mutations have been proposed. Furthermore, the oligonucleotide primers used in the PCR need to be absolutely specific and must not hybridize outside their intended targets even in minute amounts. This is achieved more easily in MHC class II genes than in class I genes, as there are fewer class II genes and the differences between different class II isotypes are much more pronounced than those between class I isotypes. An initial experiment should also involve a DNA segment known to have participated in a suspected gene conversion, as this might be a highly sequence-specific process. There is only one DNA segment that meets this description. It is a 14-bp fragment from the $E\beta^b$ gene supposedly transferred to the $A\beta^b$ gene in the bm12 mutation (11–13). To maximize the specificity of the PCR primers, we chose the $E\beta^d$ gene as donor and the $A\beta^k$ gene as acceptor, rather than the original $E\beta^b$ and $A\beta^b$ genes (Fig. 1). BALB/c was used as $E\beta^d$-bearing donor strain. C3H/HeJ and CBA were used as $A\beta^k$-bearing acceptor strains. Using donor and acceptor genes from different chromosomes was necessary also to control for *in vitro* PCR artifacts such as the one described as "jumping PCR" (23) (see below).

Controlling for *in Vitro* PCR Artifacts. Unfinished PCR products may be used as primers in the following annealing steps. If several homologous genes or alleles with a central region of identity are present in the PCR, such products may hybridize to a different but homologous DNA molecule, thus creating a hybrid molecule upon elongation. This phenomenon has been called "jumping PCR" (Fig. 2). As there are large stretches of identity between the $A\beta^k$ and $E\beta^d$ genes in the segment spanned by the primers, the risk for jumping PCR is not negligible. Such hybrid molecules would, if they appeared early during the PCR cycles, be amplified enough to create a signal indistinguishable from that of a true gene conversion event. To control for the appearance of such artifacts, for every sample from F_1 mice, a sample with a mixture of DNA from the corresponding cells from each parental strain was used at the same total DNA concentration. Thus, for every sample tube, a control was run with exactly the same DNA in the same amounts, the only difference being that the DNA from the parental strains C3H/HeJ and BALB/c or CBA and BALB/c had not been together in the same nucleus. Therefore, the controls would display the frequency of jumping PCR and other *in vitro*

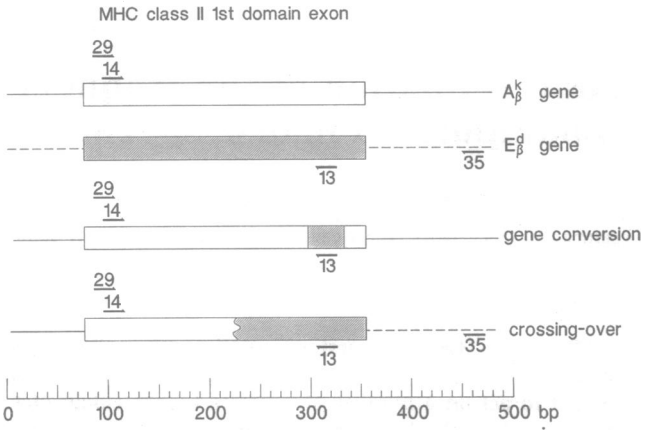

FIG. 1. Location of the primers used for the gene conversion assay and for the control assay to determine the frequency of unequal crossing-over between the $A\beta^k$ and $E\beta^d$ genes. As shown, no product will be amplified unless $A\beta^k$ sequences and $E\beta^d$ sequences have been juxtaposed to one another, as all primers of $A\beta^k$ origin are sense-directed, whereas all $E\beta^d$-derived primers have the antisense direction. The two $A\beta^k$ primers 29 (GCACCAGTTCCAGCCCTTC) and 14 (GTTCCAGCCCTTCTGCTAC) were used consecutively in two PCRs in a nested priming with the conversion donor primer 13 (CGCGCATCCTCCAGGATC) to enhance the yield and the specificity of the PCR. In the assay for unequal crossing-over, primers 29 and 35 (GAGGCTGCTTAAAAGCGCC) were used in the first PCR and primers 14 and 13 were used in the second PCR.

artifacts in the system, and any signal significantly higher than that has to be attributed to genetic *in vivo* mechanisms. The true frequency of the gene conversion event would then be obtained by subtracting the frequency of control events from the frequency of events found with F_1 sperm DNA.

Choice of Source Tissues. We chose to analyze DNA from sperm, as meiotic germ-line cells, and from liver, as representatives of somatic cells. As the frequency of a specific intergenic gene conversion event can be expected to be very low, we first performed a titration with increasing amounts of DNA shown in Table 1. Despite the relatively low number of samples used, it is evident that signals are detected, that they increase in a roughly concentration-dependent manner, and that signals occur more often with DNA from hybrid sperm than with mixed parental DNA. We wanted a frequency of positive signals of around one-third of total samples, as this

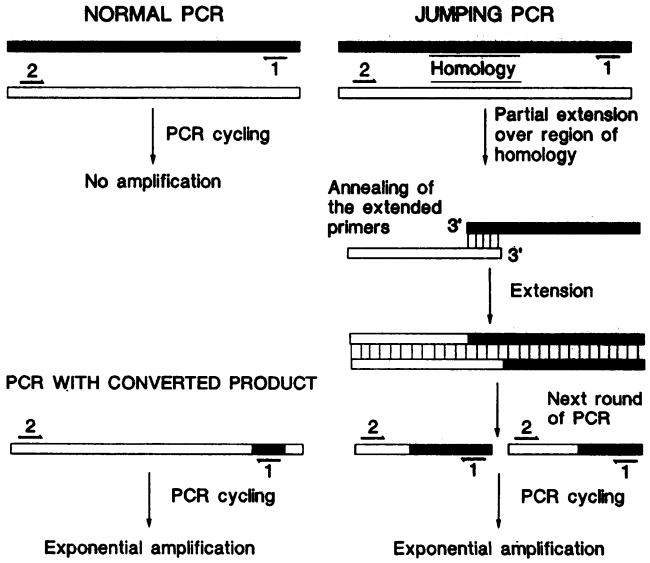

FIG. 2. Description of jumping PCR.

Table 1. Conversion events are detected in F_1 sperm in a concentration-dependent manner

Copies, no.	Mouse	Events, no. detected/ no. examined	P
42,000	(C3H × BALB/c)F_1	0/10	0
	C3H + BALB/c	0/10	0
84,000	(C3H × BALB/c)F_1	1/10	$1.25 \pm 2.5 \times 10^{-6}$
	C3H + BALB/c	0/10	0
190,000	(C3H × BALB/c)F_1	4/10	$2.69 \pm 2.9 \times 10^{-6}$
	C3H + BALB/c	0/10	0
420,000	(C3H × BALB/c)F_1	5/10	$1.65 \pm 1.7 \times 10^{-6}$
	C3H + BALB/c	1/10	$2.51 \pm 5.0 \times 10^{-7}$

DNA from sperm was amplified with the primers 29, 14, and 13 as described in Fig. 1. An event was considered as detected if a distinct band of the expected size appeared, when one-third of the second PCR products was electrophoresed on a 3% agarose gel with TBE and stained with ethidium bromide. Standard deviations were calculated regarding the gene conversion events as binomially distributed. The probability (*P*) of a detected gene conversion event per DNA molecule assuming that all events occurring are detected is also shown.

gives the highest statistical significance. Based on the values obtained in Table 1, we therefore decided that a concentration of 150,000 haploid copies of sperm DNA per PCR amplification should be used to estimate the frequency of gene conversion of this particular MHC class II gene fragment in sperm. For the somatic liver cells, where we expected no gene conversion to take place, we chose a DNA concentration of 800,000 haploid copies per cell, which was the highest DNA concentration at which we were confident that the PCR amplification would still be optimal, judging from our experience of other PCR systems. We later verified this with an artificial template (see below). We chose to analyze two haplotype k × d crosses, C3H/HeJ × BALB/c and CBA × BALB/c, to look at possible differences in gene conversion between the two strains.

Specificity of the PCR. The PCR occasionally produced fragments of an unexpected size. Such fragments were of equal abundance in the samples from F_1 sperm DNA and those from mixed parental DNA. However, all unexpected bands were clearly distinguishable from that of the expected product, which usually also was stronger. Fig. 3 shows a representative sample of PCR products from both F_1 sperm DNA and mixed parental DNA.

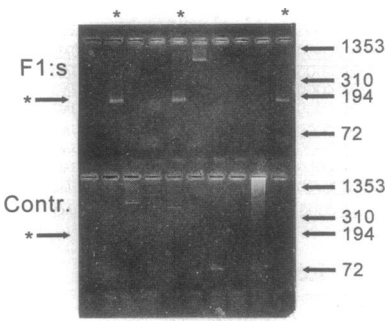

FIG. 3. Ethidium bromide staining of PCR products included in Table 2 from a 3% agarose gel. The gel has two sets of wells, one at the top and one at the middle of the gel. The dye marker has been run to a little less than half the gel's length. In the top row of wells, products from PCRs with 150,000 copies of (C3H/HeJ × BALB/c)F_1 sperm DNA and, in the bottom row, products from PCRs with a mixture of 75,000 copies each of C3H/HeJ and BALB/c sperm DNA are shown. Arrows and numbers to the right are molecular size standards in base pairs. The arrows to the left with an asterisk denote the migration of the expected 185-bp fragment. Asterisks on top of wells in top rows signify samples that were scored as positive for the expected product. F1:s, F_1 sperm DNA; contr., control.

Table 2. Frequency of detected conversion events from Eβ^d to Aβ^k

Organ	Copies, no.	Mouse	Events, no. detected/ no. examined	P
Sperm	150,000	(C3H × BALB/c)F_1	64/200	$2.57 \pm 0.64 \times 10^{-6}$
		C3H + BALB/c	2/200	$6.27 \pm 9.3 \times 10^{-8}$
		(CBA × BALB/c)F_1	50/200	$1.92 \pm 0.54 \times 10^{-6}$
		CBA + BALB/c	3/200	$1.01 \pm 1.1 \times 10^{-7}$
		All (d × k)F_1	114/400	$2.24 \pm 0.41 \times 10^{-6}$
		All d + k parental	5/400	$8.40 \pm 7.4 \times 10^{-8}$
$P(F_1) - P$(parental) = P(specific events)				**$2.15 \pm 0.42 \times 10^{-6}$**
Liver	800,000	(C3H × BALB/c)F_1	8/200	$5.10 \pm 3.6 \times 10^{-8}$
		C3H + BALB/c	11/200	$7.07 \pm 4.2 \times 10^{-8}$
		(CBA × BALB/c)F_1	11/200	$7.07 \pm 4.2 \times 10^{-8}$
		CBA + BALB/c	16/200	$1.04 \pm 0.51 \times 10^{-7}$
		All (d × k)F_1	19/400	$6.08 \pm 2.7 \times 10^{-8}$
		All d + k parental	27/400	$8.74 \pm 3.3 \times 10^{-8}$
$P(F_1) - P$(parental) = P(specific events)				**$-2.65 \pm 4.3 \times 10^{-8}$**

Amplification and analysis of products and results were as in Table 1. Final values, corrected for *in vitro* artifacts, are in boldface type. The probability (*P*) of a detected gene conversion event per DNA molecule by assuming that all events occurring are detected is also shown.

Frequency of Gene Conversion Events in Sperm. Table 2 shows that the two k × d hybrids do not differ significantly from one another and that the probability of unspecific "jumping PCR" or other *in vitro* artifacts is more than an order of magnitude lower than the signal detected in the samples with hybrid sperm DNA. As the results from the two mouse strains do not differ significantly, they were pooled to obtain a more reliable estimate. As follows from Table 2, 114 of a total of 400 PCRs with hybrid sperm DNA tested gave a *bona fide* signal, which gives a probability of detecting an event in a given tube of 0.285 ± 0.044. As there are 150,000 donor and acceptor molecules in each tube, this means that the probability per DNA molecule of a detected event is $1 - [(1 - 0.285)^{(1/150,000)}] = 2.24 \times 10^{-6} \pm 0.41 \times 10^{-6}$. In the PCRs with mixed parental DNA, 5 of 400 PCRs were positive, giving a frequency of unspecific signals of $8.40 \times 10^{-8} \pm 7.4 \times 10^{-8}$. If the probability of unspecific signals is subtracted from the total frequency in hybrid sperm, we get $2.15 \times 10^{-6} \pm 0.42 \times 10^{-6}$ as the corrected frequency for gene conversion of the Eβ^d gene DNA segment onto an Aβ^k gene in d × k sperm.

Frequency of Gene Conversion Events in Liver. In liver, the frequency of unspecific reactions per molecule was about the same as in sperm. As we had more than five times more DNA in the reaction mixtures, the frequencies per tube are considerably higher than in sperm DNA. Again, no significant differences appear between the two mouse strains. No sign of gene conversion could be detected in the liver DNA. Indeed, there were slightly fewer detections in the hybrid samples than in the mixed parental samples, which makes the corrected value for specific gene conversion $-2.65 \times 10^{-8} \pm 4.28 \times 10^{-8}$; thus, that the frequency of gene conversion per DNA molecule in liver DNA is $<1.63 \times 10^{-8}$ with 95% confidence. In other words, the frequency of gene conversion events in ordinary somatic cells seems to be at least two orders of magnitude lower than in sperm, at least for this DNA fragment. As liver F_1 DNA has undergone as much recombination as sperm F_1 DNA, this measurement also verifies that potentially recombined F_1 DNA does not have a higher frequency of PCR *in vitro* artifacts than the mixture of unrecombined parental DNA.

Test for Unequal Crossing-Over. There is one additional explanation that is theoretically possible for our results

presented so far. As our signal is the amplification of a DNA molecule with both the $A\beta^k$ and the $E\beta^d$ sequences present with the appropriate spacing, an unequal crossing-over between the $A\beta^k$ and the $E\beta^d$ gene could produce the same result. We therefore designed an additional $E\beta^d$ primer to test this hypothesis. Primer 35 (see Fig. 1) hybridizes to a sequence ≈150 bp into the intron 3′ to the first domain exon. Any crossing-over products would be amplifiable in a nested PCR using this primer and primer 29 in the first reaction and primers 13 and 14 in the second reaction. We detected no signal in 200 samples of 150,000 sperm, neither with hybrid sperm nor with mixed parental sperm (data not shown), although we could detect a high proportion of single-copy artificial templates (see below). Therefore, we conclude that unequal crossing-over plays a negligible role as the origin of events that we detect. We also can confirm that all gene conversion events detected were from $E\beta^d$ to $A\beta^k$, as any detected gene conversion in the other direction would have given a signal in this assay.

Efficiency of the PCR Assay. After all the PCR data from sperm and liver had been collected, we proceeded to verify the efficiency of our PCRs. We added an average of 0.3 copy of a hybrid $A\beta^k$–$E\beta^d$ molecule to samples with 150,000 copies of mixed parental sperm DNA as in the *in vitro* PCR artifact control above. We then performed 100 nested PCRs with the "conversion primers" 29, 14, and 13 and 75 with the "crossing-over primers" 29, 14, and 35, exactly as described above. Twenty-seven reactions with the "conversion primers" and 16 reactions with the "crossing-over primers" yielded the expected product on an agarose gel. As 26% of the samples would be expected to give a signal at 100% efficiency in the PCRs, we concluded that our nested PCR could amplify a very high proportion of single DNA molecules with both sets of primers. We also tested increasing concentrations of liver DNA plus 0.3 copy of hybrid template. We obtained the expected frequencies in liver DNA with between 300,000 and 800,000 copies per reaction mixture. However, at 1,000,000 copies, the frequency decreased ≈3-fold.

Sequences of Converted PCR Products. To investigate the size of the fragments transferred, we have sequenced a sample of the positive PCR products. Since the amplification had to be performed with a donor sequence primer, only one breakpoint can be inferred. As shown in Fig. 4, the length of a fragment transferred by gene conversion is not fixed but can vary. The total length of DNA transferred from $E\beta^d$ to $A\beta^k$ cannot be determined, as we can assay only one breakpoint. We note, however, that the minimal amounts of DNA transferred, if the 3′ breakpoint is immediately downstream primer 13, are 38, 63, and 104 nt. The original bm12 mutation contained between 14 and 44 nt (12, 13). It therefore seems conceivable that all the mutations sequenced represent a transfer of DNA considerably longer than that of the bm12 mutant. This might in part be due to sequence differences. The $A\beta^k$ and $E\beta^d$ alleles were chosen to obtain maximum divergence. In particular, there is a 6-nt deletion, shown in Fig. 4, of the $A\beta^k$ gene with respect to $E\beta^d$ just upstream to the primer 13. Perhaps this difference in length selects against breakpoints near this primer, as most breakpoints seem to be within stretches of >20 nt of sequence entirely similar between the donor and acceptor gene. We also want to point out that on the order of 5% of the fragments we amplify from F_1 sperm DNA should come from *in vitro* PCR artifacts. Therefore, there is an ≈35% chance that a recombined sequence has arisen in this manner in Fig. 4 and a slight risk that a sequence present only once, such as the one implying the longest fragment transferred, has not actually arisen by gene conversion.

DISCUSSION

The rate of gene conversion in higher eukaryotes has previously been studied with two methods. One method employs the study of drug resistance in cultured cells transfected with two complementary defective copies of a drug resistance gene. This method has been used twice. In both cases, the assay was expression of drug resistance in cultured cells. In one case with the neomycin-resistance gene in NIH 3T3 cells, frequencies of $\approx 10^{-6}$ were detected for a gene conversion event that restored neomycin-resistance gene activity (14). In the other case with the herpes simplex virus *tk* gene in L cells, the detected frequency of gene conversion was between 10^{-6} and 10^{-8} in various experiments (15). It is difficult to tell the relevance of these results for the *in vivo* frequency of gene conversion. They were obtained in cultured somatic cells, rather than in the germ cells of a living organism and were assessed on transfected genes totally alien to the cells.

The other method involved the use of transgenic mice. Murti *et al.* (16) created transgenic mice carrying two tandem copies of complementary defective *lacZ* genes under a promoter active in sperm and assayed the gene conversion by looking at the number of blue-stained sperm. They obtained a much higher frequency; ≈2% of the sperm of transgenic

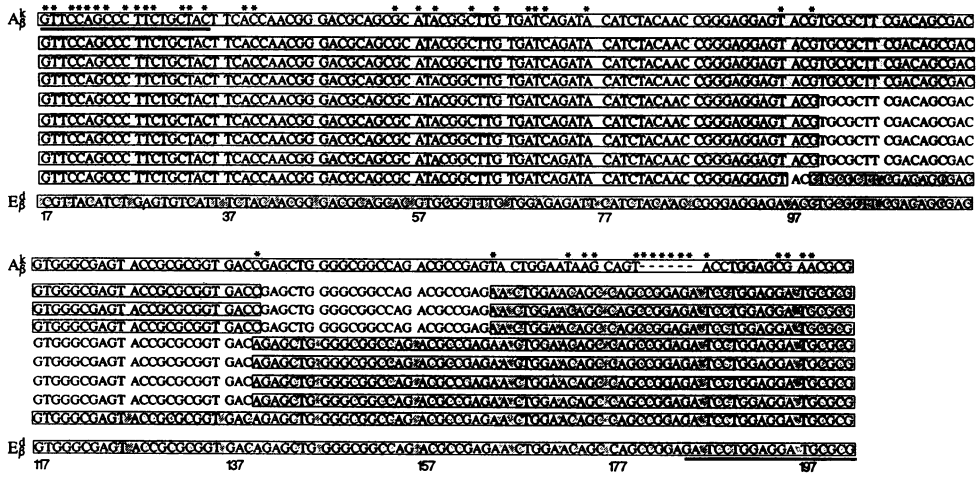

FIG. 4. Sequences of eight PCR products from the assay described in Fig. 1 and Table 1. On top, the $A\beta^k$ sequence is shown. At the bottom the $E\beta^d$ sequence is given. Shaded boxes indicate identity with $A\beta^k$. Hatched boxes denote identity with $E\beta^d$. PCR product sequences, neither boxed nor shaded, represent stretches where the $A\beta^k$ and $E\beta^d$ sequences are identical, and it cannot be determined whether they are of $A\beta^k$ and $E\beta^d$ origin. Numbers start at the first nucleotide of the first domain exon of the $E\beta^d$ gene, and asterisks mark the dissimilar residues in $A\beta^k$ and $E\beta^d$.

mice were stained. This experiment is obviously much closer to nature than experiments on cultured cells. However, it still involved genes from a kingdom other than that of the host organism and, perhaps more importantly, it involved two extremely similar genes in tandem with virtually no other DNA between them. It seems therefore reasonable to assume that the experiment has measured the absolute maximal rate of gene conversion, rather than the rates actually present between similar genes in an unmanipulated *in vivo* situation.

The rate of gene conversion that we find in this communication is several orders of magnitude lower than that of Murti *et al.* (16). There is plenty of reason to believe that the particular gene conversion event we chose to detect is a rare event, as gene conversions go. It involves a gene conversion between two genes, as opposed to alleles, one on the paternal and one on the maternal chromosome. Furthermore, it involves, for reasons outlined above, MHC class II genes rather than class I genes. To date, only one such conversion mutant has been found phenotypically in mice, whereas ≈30 class I mutants have been found in the same kind of screenings. It is therefore conceivable that gene conversion between different class I genes or interallelic gene conversion of class II genes, as in the human *DPB* alleles, might have higher frequencies than those reported here. Around 100,000 mice have been screened for bm mutations (24). The total frequency of one class II conversion mutant in 100,000 mice is not obviously incompatible with our frequency of one specific mutant in 500,000 sperm.

Whenever PCR detection, and in particular single-copy detection, is concerned, utmost care has to be taken to eliminate the possibility of contamination as the cause of the results. We are confident that the results presented were not caused by contamination for several reasons. First, we maintained a rigorous separation of the different steps involved in the assay. Preparation of reagents, pipetting the ingredients of the first PCR, pipetting the ingredients of the second PCR, and analysis of the final products were carried out in four widely separated rooms, with different designated sets of pipettes. It is also highly improbable that we should have been able to contaminate only the samples from F_1 sperm and not other DNA. Seven DNA preparations from seven mice were used for the F_1 sperm results in Tables 1 and 2, and there was no significant variations between the various DNA preparations. However, the strongest argument against contamination as the source of the results comes from the fact that we amplified a sequence that had never been cloned or amplified before. Our first positive results simply had no possible contaminating template. As we started to obtain positive results, we introduced a theoretical risk of contamination. Still, the frequency did not rise from the first preliminary titrations in Table 1 to the later experiments in Table 2.

In this communication, we find evidence that the bm12 mutation repeats itself, from a haplotype d donor gene onto a haplotype k acceptor gene, on the average twice in every million sperm, a cell where no selection of MHC class II gene function is likely to have occurred. We consequently feel that we have put the question whether gene conversion-like events really do occur in the genes of the major histocompatibility system to rest. Even if the frequency that we detect is low, it is sufficient to explain the observed frequency of apparent conversion mutations for class II genes. We have also indicated that DNA stretches longer than those previously suggested can be transferred by gene conversion in the mouse MHC. The method we employ can in principle be used to detect all gene conversion between nonidentical genes. It remains to be investigated how many different fragments can be transferred in this way and which genes can serve as acceptor genes. It also remains to be investigated whether this process occurs at detectable frequencies in some mitotic cells, such as B lymphocytes, where gene conversion has been proposed for immunoglobulin genes (3, 17, 18). We are furthermore aware that this assay might enable us to pinpoint the exact cell stage at which gene conversion occurs and, eventually, to identify gene products responsible for the phenomenon.

We thank Lena Israelsson and Zekiye Cansu for technical assistance, Gunnel Jansson and Solveig Sundberg for animal care, Helene Olsson for help with the computerized gel-reading system, and Anna Fogdell for teaching us her DNA sequencing protocol and for being generally helpful. This research was supported by Grant B94-13C-10835-01 from the Swedish Medical Research council to J.B.

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