Having demonstrated the specificity of the *Mycoplasma* rDNA probe, we adapted a dot-blot hybridization procedure (*13*) for the detection of mycoplasma infection in tissue culture using the nick-translated 900-bp Hind III fragment of M13Mh129 as probe. The assay detected less than 0.5 pg of homologous DNA (Fig. 4A). This corresponds to the amount of rDNA contained in less than 1×10^5 mycoplasmas, assuming the presence of one rRNA operon in a genome of about 800 kilobase pairs (kbp) in size. We obtained similar values by blotting a suspension of mycoplasma-infected cells onto nitrocellulose filters. As shown in Fig. 4B, fewer than 1×10^5 mycoplasmas could be detected.

The results obtained with probe M13Mh129 show that it is specific for mycoplasmas and that the detection assay is quantitatively sensitive, ranking with the most sensitive indirect methods (*14*). Depending upon the conditions of hybridization, the probe may be used to detect any prokaryotic organism (because of the conserved nature of ribosomal RNA) or specifically mycoplasmas. It is not possible to distinguish between groups of microorganisms in this fashion with DNA-binding dyes. Immunofluorescence is the most specific technique for detecting mycoplasmas. However, unless the antibodies recognizing the particular contaminating mycoplasma are used in the test, a false negative result is obtained. The *Mycoplasma* rDNA probe recognizes a sequence that is conserved among various genera.

Various technical refinements may also improve the efficiency of this probe. For example, DNA-RNA hybridization (*15*) should significantly amplify the signal and provide an even more sensitive probe since each mycoplasma cell contains only one or two rRNA operons but dozens of ribosomes. The use of a nonradioactive detection scheme, for example, tagging the DNA probe with biotinylated nucleotides (*16*), might also improve the application of this technique and make it more versatile.

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Homologous Recombination Catalyzed by Mammalian Cell Extracts in vitro

Abstract. *An assay was developed to detect recombination events taking place in an in vitro reaction. Extracts of cultured mouse preB lymphocytes were found to catalyze homologous recombination between substrate DNA molecules but not site-specific recombination between cloned mouse immunoglobulin D and J genes. Addition of deoxyribonucleoside triphosphates increased the frequency of homologous recombination. This recombination activity was not observed in two differentiated lymphocyte cell lines.*

DNA rearrangements mediated either by site-specific or homologous mechanisms can play a role in the regulation of gene expression. Of the various organisms in which these rearrangements have been found, only in bacteria and bacteriophages have the molecular mechanisms been elucidated (*1*). The use of in vitro systems has played a vital role in their study and has recently been extended to the lower eukaryote yeast (*2*). We now report the development of an in vitro system in a higher eukaryote, the mouse. We used cells of the B-lymphocyte lineage in which the assembly of immunoglobulin genes from genomic DNA segments takes place (*3*). We have constructed DNA substrates with which to distinguish several types of recombination at various stages of development. With this system, we have detected efficient homologous recombination between exogenously added DNA substrates (*4*), but have not yet been able to demonstrate the site-specific recombination known to occur between D and J components of immunoglobulin (Ig) variable regions (*3*). We have also determined some of the biochemical requirements of the homologous recombination system.

Table 1. Frequency of homologous recombination in vitro. Recombinants were assayed by counting Spi^- plaques on the selective host Q364 or am^+ plaques on the selective host W3350 Su^0. Total phage were assayed on non-selective hosts K802 (for Spi experiments) and Ymel Su III (for amber experiments). Recombination frequency was calculated relative to the total number of phage packaged, to allow for variable DNA recovery.

Experiment	Substrates	Recombination frequency	
		Control*	38B9†
1	Fig. 1a	1.0×10^{-5}	1.0×10^{-3}
2	Fig. 1b	2.0×10^{-5}	1.9×10^{-4}
3	Fig. 1c	1.2×10^{-5}	4.2×10^{-4}
4	Fig. 1d	4.5×10^{-4}	2.2×10^{-2}
5	Fig. 1d	3.2×10^{-3}	7.4×10^{-2}
6	Fig. 1d	9.0×10^{-4}	2.7×10^{-3}
7	Fig. 1e	1.0×10^{-4}	7.0×10^{-2}
8	Fig. 1e	3.6×10^{-5}	1.8×10^{-2}

*This is the control without extract; the substrates were mixed before packaging. †In this set of experiments the substrate was incubated with 38B9 before packaging.

In our in vitro reaction, extracts of nuclear proteins from cultured mouse cells were incubated with bacteriophage λ DNA molecules carrying recognizable genetic markers. The phage DNA was then recovered from the reaction, purified, and packaged in vitro into infective capsids and assayed for recombination by plating on appropriate selective host strains.

The phage substrates contained either mouse Ig gene segments complete with their nonamer-heptamer site-specific recombination signal sequences (*3*) or, alternatively, phage amber mutations but no mouse DNA. The principles of the assays are as follows: (i) Loss of the phage λ marker "Spi" from between the mouse D and J segments can be detected by a simple plating test since Spi^+ phage cannot plate on a host that is lysogenic

Table 2. In vitro recombination promoted by extracts of different cell lines (*16*). These experiments were performed with Spi selection. The substrates were those shown in Fig. 1a.

Extract from		Estimations (No.)	Activity in vitro	Recombination frequency
Cell line	Cell type			
38B9	Pre-B[a]*	8	+	1.3×10^{-3}
B6Mo	Early T[b]†	3	+	6.8×10^{-4}
1881	Pre-B	10	+	5.0×10^{-4}
4E4 11	Pre-B	4	+	1.9×10^{-4}
3T3	Fibroblast‡	2	+	$[1.8 \times 10^{-4}]$
PD31	Pre-B	5	+/−	1.5×10^{-4}
2A	T	2	−	3.0×10^{-5}
NSI	Plasmacytoma	2	−	2.0×10^{-5}
No extract		>20	−	1.0×10^{-5}

*The pre-B cell lines were Abelson virus-transformants fixed very early in development. †B6Mo is transformed by Moloney virus, also probably fixed very early but in the T-cell lineage. ‡The recombinants produced by 3T3 extracts contained no mouse DNA and were not produced by a homologous process.

for the unrelated phage P2 (*5*). Thus, site-specific recombination joining D to J would remove the Spi segment allowing the recombinants to be selected by growth on the *Escherichia coli* host strain Q364 (a P2 lysogen). (ii) If a second substrate phage is added to the reaction, with Ig genes in a different configuration, the Spi$^-$ selection reveals the loss of Spi by homologous recombination (Fig. 1, a to c) when homology between the D and J segments on separate phages mediates recombination. These two types of recombinants can be distinguished by mapping. Site-specific D-J joining deletes intervening mouse sequences as well as Spi, whereas homologous recombination removes only the Spi segment and restores the Ig genes to their intact germline configuration (*3*).

For detection of single crossovers mediated by homologous recombination, we used pairs of phage amber mutants. If two phages containing amber mutations in different genes undergo a crossover anywhere between them, the wild-type recombinants can be selected on the *E. coli* Su^0 host (Fig. 1 and Table 1). In the experiments shown, extracts were prepared from cell line 38B9 (*6*) and incubated with the DNA substrates diagramed in Fig. 1. (*7*).

When two Ig-containing substrates were used together in the in vitro reaction, significant numbers of recombinants were obtained (Table 1). Analysis of these recombinants by restriction mapping, blotting, and hybridization (*8*) showed that they were formed by a homologous process that deleted Spi but left the Ig segments and their intervening sequences intact (confirmed by DNA sequencing). Double-stranded break-and-join events were clearly taking place in these reactions, since a simple repair process would not have removed Spi. The presence of the lac gene in the recombinants confirmed the double crossovers shown in Fig. 1, a and b.

In these experiments, site-specific recombinants between D and J would also have been detected as Spi$^-$ phages in the plating assay, but none were found. No D-J recombination was observed in single substrate reactions either (data not shown).

Experiments with amber mutant phages lacking mouse genes are presented in Fig. 1, d and e. Recombinants formed by a single crossover in the central region of the phage were observed at very high frequency, up to 7×10^{-2} (Table 1). Simple repair processes were not responsible for this effect since incubation of the amber substrates one at a time did not produce wild type (data not shown). The recombinants were further characterized by plating tests for unselected markers cI and lac5 indicating that the breakpoints were more or less randomly distributed between the amber mutant sites (data not shown).

By altering the conditions of the in vitro reaction, we have established that Mg^{2+} is required at 2 to 4 m*M* for efficient recombination, and that addition of 0.25 m*M* deoxynucleoside triphosphates (dNTP's) stimulates the frequency of recombination by a factor of 8 (three experiments). This suggests that a DNA polymerase may be involved such as has been proposed generally (*9*) and more specifically for recombination in yeast (*10*).

Two control experiments showed that recombination was actually taking place in the cell extract and not in the bacterial extracts used in the in vitro packaging reaction. (i) When the two substrate phage DNA's were mixed together without exposure to cell extracts very little recombination resulted (Table 1); and (ii)

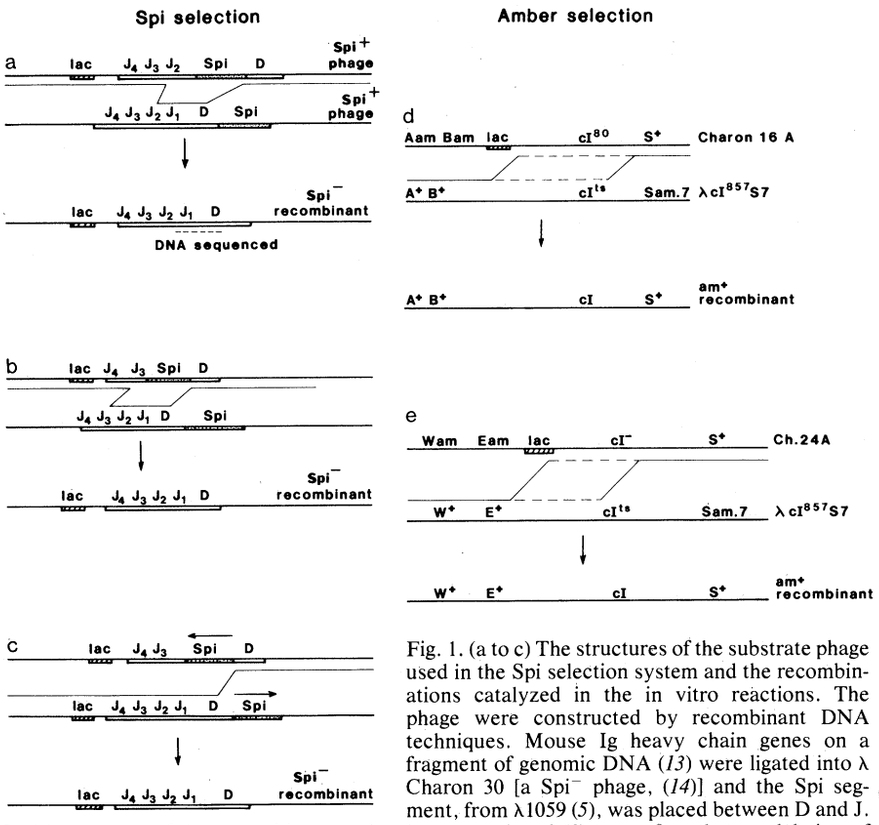

Fig. 1. (a to c) The structures of the substrate phage used in the Spi selection system and the recombinations catalyzed in the in vitro reactions. The phage were constructed by recombinant DNA techniques. Mouse Ig heavy chain genes on a fragment of genomic DNA (*13*) were ligated into λ Charon 30 [a Spi$^-$ phage, (*14*)] and the Spi segment, from λ1059 (*5*), was placed between D and J. In (a) a portion of the recombinant molecule was analyzed (*8*) to confirm that no deletion of mouse DNA had occurred between D and J_2. The sequence data for the marked area was identical with the germline sequence. (d and e) Pairs of amber mutant phages that produced am$^+$ recombinants when incubated together with cell extracts. The λ Charon phages 16A (Aam and Bam) and 4A from which Ch24A (Wam and Eam) was derived have been described (*15*). Abbreviations: Aam, A amber; Bam, B amber; Eam, E amber; and Wam, W amber.

when substrate phage DNA's were incubated separately with cell extracts, repurified, and then mixed before packaging and plating, no recombinants were obtained either (<1 percent of the maximum yield). Thus the recombination event must have taken place in the mammalian cell extracts and not the packaging preparations. These controls were particularly important since it has been reported that recombination between Ig heavy chain switch sites (not present in our constructs) can occur during packaging (*11*) even when packaging extracts were made (as ours were) from bacteria whose recombination systems had been inactivated by *red* and *recA* mutations.

Several different murine cell lines were tested for recombination activity (Table 2) including early B- and T-cell lines. A myeloma line was included in which genome rearrangements are presumed to have ceased, as well as a mature T-cell and a fibroblast line. These might be expected to be negative for recombination activity. The cell lines showing the highest levels of activity, 39B9 and 1881, are known to rearrange their Ig genes in culture, whereas the fully differentiated T-cell and myeloma lines in which DNA rearrangements do not occur showed no activity. The fibroblast line did produce low but significant levels of Spi$^-$ recombinant candidates, but they contained large deletions; no homologous or exact D-J recombinants were found.

The fact that homologous recombination activity is most efficient in cell lines that can actively recombine Ig gene segments and negative in those that do not suggests that we may be observing a partial recombination activity with a role in Ig gene expression. The high level of activity in the early T-cell extracts may also be consistent with this idea, since the genes for T-cell receptor polypeptides are assembled from dispersed segments by a process very similar to that for Ig genes (*12*).

Our results show that the in vitro system can be exploited in the study of mammalian recombination with engineered substrates, but suggest caution in correlating in vivo with in vitro effects.

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6. Cells were cultured in RPMI 1640 medium containing 10 percent fetal calf serum and antibiotics. The cells (5×10^7 to 5×10^8) were harvested, washed in serum-free medium, and resuspended in 2 ml of 10 m*M* tris-HCl at *p*H 7.4, 10 m*M* $MgCl_2$, 10 m*M* KCl, and 1 m*M* dithiothreitol. Phenylmethyl sulfonyl fluoride (1 m*M*) was added just before homogenization in a Dounce glass homogenizer. Nuclei were collected by centrifugation at 1000 rev/min in a clinical centrifuge and resuspended in 1 ml of the same buffer. NaCl was added to a concentration of 0.3*M*, and the mixture was incubated on ice for 1 hour. The extract was then centrifuged at 100*g* (Beckman 50Ti rotor), and the supernatant was dialyzed. Portions were frozen in a mixture of CO_2 and ethanol and stored at −80°C.
7. Generally, 10 μg of substrate DNA was incubated with 15 μg of cell extract. The reaction also contained 10 m*M* tris-HCl (*p*H 7.5), 5 m*M* $MgCl_2$, 0.5 m*M* adenosine triphosphate (ATP), and ribonuclease (100 μg/ml) in a total volume of 100 to 150 μl. After 1 hour at 37°C the in vitro reaction was terminated by addition of sodium dodecyl sulfate and proteinase K (to 0.5 percent and 0.25 mg/ml, respectively) and incubated for 30 minutes at 37°C. Phage DNA was purified by several phenol and chloroform extractions, and finally by ethanol precipitation. The phage DNA was then packaged in vitro into capsids, and plated on appropriate host bacteria. In vitro packaging extracts were made from the lysogens BHB2688 and BHB2690 [B. Hohn and K. Murray, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3259 (1977)]. The protocol was a modification of that described by B. A. Zehnbauer and F. R. Blattner [in *Genetic Engineering*, J. K. Setlow and A. Hollaender, Eds. (Plenum, New York, 1982), vol. 4, pp. 249–279].
8. Restriction digests of DNA samples were subjected to electrophoresis on agarose gels and then blotted on to nitrocellulose. Plasmid subclones of D, J, and Spi fragments (in pBR322 derivatives) were labeled with ^{32}P by nick translation and hybridized to the gel blots; hybridizing bands were then detected by autoradiography; the DNA sequences were determined by the method of A. Maxam and W. Gilbert [*Methods Enzymol.* **65**, 499 (1980)].
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Morphine Analgesia Potentiated but Tolerance Not Affected by Active Immunization Against Cholecystokinin

Abstract. *Administration of cholecystokinin was recently found to attenuate opiate analgesia. In the present study, the role of endogenous cholecystokinin in opiate analgesia was examined. Endogenously released cholecystokinin was sequestered by antibodies to cholecystokinin developed in response to an active immunization procedure. Morphine analgesia was potentiated and prolonged in rats immunized against cholecystokinin. The rate of development of morphine tolerance, however, was not affected by the antibodies. Endogenous cholecystokinin appears to function as a short-term modulator of opiate action.*

Accumulating evidence supports the hypothesis that there is a physiological antagonism between cholecystokinin (CCK) and opiates (*1*). The sulfated octapeptide variant of CCK potently and specifically attenuates opiate-mediated forms of analgesia produced by foot shock (*1*), morphine (*1*), and β-endorphin (*2*). Other opiate-dependent processes, including tail pinch–induced feeding (*3*) and suppression of thyrotropin-releasing hormone–induced wet dog shakes by β-endorphin (*4*), are also antagonized by CCK octapeptide. In addition to these experiments with exogenous CCK, indirect evidence supports an involvement of endogenous CCK in nociception. Anatomically, CCK is present in areas of the central nervous system (CNS) known to modulate the intensity of pain perception, such as the periaqueductal gray region and the dorsal horn of the spinal cord (*5*). Also, the finding that levels of CCK in the brain decrease in response to systemically administered morphine (*6*) indicates a functional interaction between CCK and opiate systems. These findings, along with the observation that several other effects of CCK octapeptide are the opposite of those reported for opiates (*1*), suggest that an opiate-antagonistic function of CCK underlies several actions previously ascribed to this peptide.

If endogenous CCK does indeed function to inhibit opiate-dependent mechanisms, then blockade of CCK's action (*7*) should potentiate or prolong the effects