brain LDCV's seem analogous to those of secretory granules of endocrine cells (*18, 22, 24*). In contrast, SSV's—for example, secretory vesicles that are continuously recycled without involvement of the central "headquarters" of the cell—do not have an equivalent in nonneuronal cells. Thus the presence of synapsin I only in neurons may be related to the fact that these secretory vesicles are present only in neurons and are absent from other secretory cells.

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14. The term "dense-core vesicle" has been used to define two types of vesicles in nerve endings [R. L. Klein, H. Lagercrantz, H. Zimmermann, Eds., *Neurotransmitter Vesicles* (Academic Press, New York, 1982). One type, the small dense-core vesicle, acquires an electron-opaque core under certain fixation conditions and has a diameter of 40 to 60 nm. The dense core is thought to represent a catecholamine-containing precipitate. We consider this type of vesicle to be part of the SSV population. The other type, the large dense-core vesicle, is larger and has an electron-opaque core irrespective of the fixation procedure used. These vesicles generally appear to contain a variety of secretory peptides (chromogranins, peptide neurotransmitters, and so forth) instead of or in addition to a classical neurotransmitter. The term "LDCV" refers to the latter vesicle population.
15. Bovine hypothalami were gently homogenized in four volumes of ice-cold 0.25*M* sucrose in a glass and Teflon tissue grinder (clearance, 0.10 to 0.15 mm). Homogenates were then fixed under isotonic conditions (nonlytic type B fixation) or hypotonic conditions (lytic fixation) (*3*) and embedded in agarose (*3*). This technique, in which nerve endings are disrupted in solutions of low ionic strength by mechanical shearing forces (homogenization) or by hypotonic treatments before fixation and agarose embedding, was designed to maximize labeling of cytoplasmic membrane-associated antigens. Virtually identical labeling patterns were obtained in disrupted nerve endings with isotonic and with hypotonic fixation. Total homogenates rather than crude synaptosomal fractions were used in our study to avoid a possible loss of selected nerve terminals during synaptosomal preparation. The hypothalamic region of the brain was chosen because of its high content of peptide neurotransmitters and LDCV's. Immunolabeling was carried out as described (*3*), with the exception that the Protein A–gold complex (5 nm) [prepared according to J. W. Slot and H. J. Geuze, in *Methods in Neurosciences*, C. Cuello, Ed. (Wiley, New York, 1983), vol. 3, pp. 323–346], was used as a probe to detect bound rabbit immunoglobulin G's (IgG's) directed against synapsin I, and that control goat IgG's were omitted from solution A.
16. The ratio of LDCV's to SSV's appeared to be somewhat lower in ruptured than in sealed nerve endings, as if LDCV's were preferentially lost upon lysis of the ending (Fig. 1A).
17. Random electron micrographs (×30,000) of nerve endings containing at least one LDCV were taken. Sections of immune and control preparations had similar thicknesses. Negatives were printed at a final magnification of ×112,500. A line was drawn parallel to portions of SSV's and LDCV's not contiguous to other structures at a distance 250 Å from the vesicle membrane. This distance was selected since synapsin I may partially protrude from the vesicle surface and since 150 Å is approximately the distance to be expected between the colloidal gold particles and the antigenic site [see J. W. Slot and H. J. Geuze, in (*15*)]. Gold particles present in the space between this line and the vesicle surface were counted. Corresponding vesicle profiles were counted with a Zeiss MOP 1 quantitative digital image analyzer. The total length of vesicle profiles was 31 μm for SSV's and 5.5 μm for LDCV's. The data represent ratios of total number of gold particles to total length.
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Cloned Mycoplasma Ribosomal RNA Genes for the Detection of Mycoplasma Contamination in Tissue Cultures

Abstract. *A cloned fragment of the mycoplasma ribosomal RNA operon was used as a molecular probe for the detection of mycoplasmas in cell cultures. According to the conditions of hybridization, the probe can detect prokaryotes in general or mycoplasmas specifically.*

Mycoplasmas are important tissue culture contaminants that exist in close association with the host cell membrane (*1–3*). This infection causes structural damage and changes in host cell metabolism which can interfere with experiments or result in the loss of the infected cell line (*4, 5*). Constant monitoring of cells is therefore imperative (*6*). Several techniques for the detection of mycoplasma contamination have been developed, including cultivation in appropriate media, immunofluorescence, or measurement of uridine-uracil incorporation ratio, and fluorescent staining by DNA-binding dyes such as diaminophenyl indole (DAPI) and Hoechst 33258 (*7*). We describe here a new mycoplasma detec-

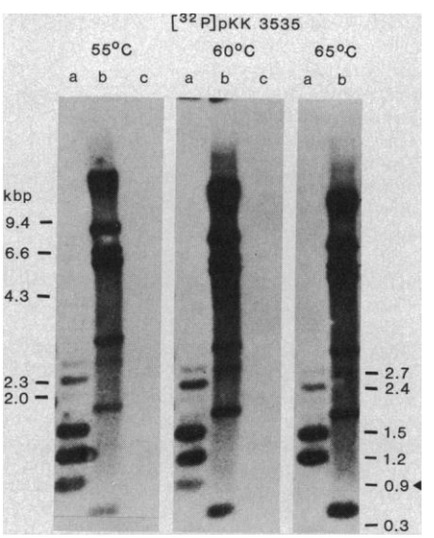


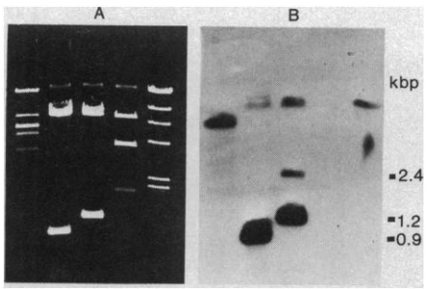
Fig. 1. Identification of rRNA gene fragments in Hind III–digested *M. hyorhinis* DNA by Southern blot analysis. DNA's (2 μg) were digested with Hind III, subjected to agarose gel electrophoresis, and transferred onto nitrocellulose filters (*16, 17*). The filters were baked at 80°C for 2 hours at reduced pressure and hybridized to nick-translated ^{32}P-labeled pKK3535 probe (specific activity 2×10^8 to 4×10^8 cpm/μg). (Lane a) *M. hyorhinis* DNA; (lane b) *E. coli* DNA; and (lane c) HeLa DNA, digested with Hind III. The lengths of the Hind III–digested lambda DNA markers and the six Hind III fragments of the *M. hyorhinis* rRNA operon are indicated in kilobase pairs. The 300-bp fragment is only seen by overexposing the film. The 900-bp *M. hyorhinis* 23*S* rDNA fragment is marked by an arrowhead.

tion assay, based on DNA-DNA hybridization, with cloned fragments of the ribosomal RNA (rRNA) operon of *Mycoplasma hyorhinis* as hybridization probes. The probe discriminates between mycoplasma and the eubacterium *Escherichia coli*. This test combines the sensitivity of DNA-staining dyes with the specificity of immunofluorescent techniques. The probe should detect all known *Mycoplasma* species as it is derived from a part of the rRNA operon which appears to be highly conserved in the genus *Mycoplasma*.

Since there are significant differences in base composition and electrophoretic mobility between mycoplasmal and bacterial rRNA's (*8–10*), we attempted to identify mycoplasmal DNA sequences that might account for these differences. A plasmid (pKK3535) (*11*) that contains the entire rrnB operon of *E. coli* hybridized to six bands in Hind III–digested *M. hyorhinis* DNA (Fig. 1). One of these bands, representing a 900-base-pair (bp) fragment from the 5′-terminal region of the *M. hyorhinis* 23*S* rRNA gene (*12*), disappeared when the hybridization was performed at higher temperatures, indicating a lower degree of homology between this particular fragment and the *E. coli* rrnB operon.

For further analysis, Hind III–digested *M. hyorhinis* DNA was cloned into the bacteriophage M13. Two clones, M13Mh129 and M13Mh171, contained inserts of 900 and 1200 bp in length, respectively, which hybridized to pKK3535 (Fig. 2). Mapping studies have shown that both fragments derive from the 23*S* rRNA gene (*12*). Both the 900- and 1200-bp fragments were purified and used as probes to identify rRNA gene (rDNA) fragments of representative *Mycoplasma* species: *M. arthritidis, M. fermentans, M. hominis, M. hyorhinis, M. pneumoniae*, and *Acholeplasma laidlawii*.

We found comparable hybridization among all species tested. Since the 900-bp fragment showed less homology than the 1200-bp fragment to the *E. coli* rrnB operon we did the converse experiment by hybridizing the M13Mh129 fragment to Hind III–digested *E. coli* DNA. Hind III–digested HeLa-cell DNA was included in this experiment, to determine the extent of homology between *Mycoplasma* rDNA and eukaryotic genomic and mitochondrial rRNA genes. There was substantial cross-hybridization between the *M. hyorhinis* 900-bp probe and genomic DNA fragments of the two *Mycoplasma* species included in this experiment. The extent of cross-hybridization to *E. coli* was negligible and no cross-hybridization at all was found to HeLa DNA (Fig. 3). In addition, purified nick-translated HeLa mitochondrial DNA did not hybridize to *M. hyorhinis* DNA digests transferred to nitrocellulose filters. The same result was found when nick-translated M13Mh129 was used to probe mitochondrial DNA restriction fragments immobilized on nitrocellulose filters (data not shown).



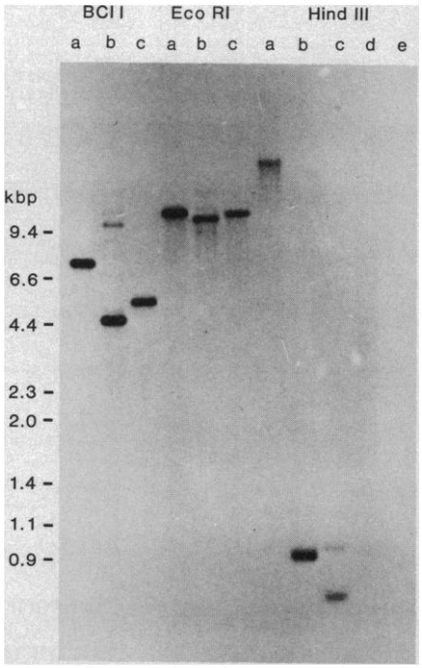


Fig. 2 (left). Cloning of *M. hyorhinis* rRNA gene fragments in phage M13. Hind III–digested *M. hyorhinis* DNA was cloned into the Hind III site of the replicative form (RF) of the bacteriophage M13mp8 (*19*). Recombinant phage–containing rRNA gene fragments were identified by Southern blot analysis (*16, 17*) with nick-translated plasmid pKK3535 as probe. Hybridization was performed as described in the legend to Fig. 1. Two positive clones were selected and RF DNA was prepared, digested with Hind III, and subjected to Southern blot analysis with pKK3535 as probe. (A) Ethidium bromide–stained agarose gel with (lane a) Eco RI–digested lambda-DNA (1.0 μg); and Hind III–digested DNA's of (lane b) M13Mh129 (0.5 μg), (lane c) M13Mh171 (0.5 μg), (lane d) M13Mh39 (0.5 μg), and (lane e) lambda (1.0 μg). M13Mh129 and M13Mh171 contain *M. hyorhinis* 23*S* rDNA; M13Mh39 contains *M. hyorhinis* nonribosomal sequences. (B) Southern blots of gel (A) with ^{32}P-labeled pKK3535 as probe.

Fig. 3 (right). Homology between the M13Mh129 insert and other DNA's. The DNA of *M. arthritidis, M. hyorhinis*, and *M. pneumoniae* was digested with Bcl I, Eco RI, and Hind III; *E. coli* and HeLa cell DNA only with Hind III. Double-stranded M13Mh129 DNA was digested with Hind III and the mycoplasma rDNA fragment purified by preparative agarose (1 percent) gel electrophoresis. The purified fragment was nick-translated to a specific activity of 5×10^8 cpm/μg. Between 1×10^6 and 2×10^6 cpm per filter were used in the hybridization assay, which was performed at 65°C as described in the legend to Fig. 1. The DNA samples tested were (lane a) *M. arthritidis* (1.5 μg); (lane b) *M. hyorhinis* (1.5 μg); (lane c) *M. pneumoniae* (1.5 μg); (lane d) *E. coli* (3.0 μg); (lane e) HeLa cells (5.0 μg).

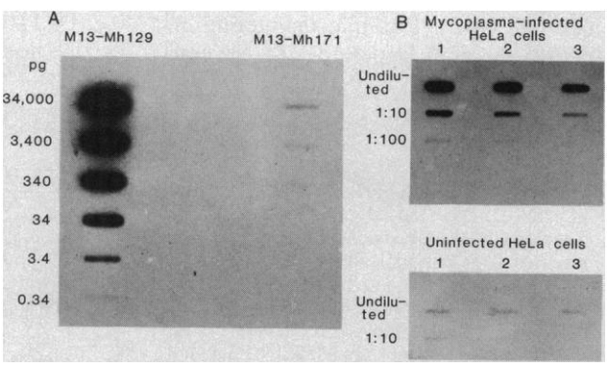


Fig. 4. Use of a cloned *Mycoplasma* rRNA gene fragment (M13Mh129) for the detection of mycoplasma contamination in tissue cultures. HeLa cells, chronically infected with *M. hyorhinis*, were tested for colony-forming units (cfu) by agar-plating assay (*14*). The same fractions were blotted onto nitrocellulose filters, denatured by alkali treatment, neutralized, and analyzed by the Southern technique (*16, 17*). (A) Single-stranded M13Mh129 and M13Mh171 DNA (270 ng to 2.7 pg, corresponding to 34 ng to 0.34 pg insert DNA) blotted onto nitrocellulose paper and probed with purified, nick-translated M13Mh129 insert (specific activity 5×10^8 cpm/μg). The slight cross-hybridization to higher concentrations of M13Mh171 DNA is due to M13 DNA contamination in the insert preparation. (B) Whole-cell blotting of serial dilutions of infected and uninfected HeLa cells. The cells used in the negative control were checked by both culturing in mycoplasma agar and by fluorescent DNA staining for the absence of contaminating mycoplasmas. The same probe was used as in Fig. 4A. For infected cells (lane 1) 2.5×10^7 cfu per 3×10^5 cells; (lane 2) 1.3×10^7 cfu per 1.5×10^5 cells; (lane 3) 7×10^6 cfu per 7.5×10^4 cells. For uninfected cells (lane 1) 2×10^5 cells; (lane 2) 1×10^5 cells; (lane 3) 0.5×10^5 cells. The number of mycoplasmas detected in this assay was between 7×10^4 and 1×10^5 cfu. The low level of hybridization found in the control slots is due to nonspecific trapping by proteins or cell debris.

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