ity is in contrast to the situation with conventional virus vaccines (*15*). Furthermore, the levels of neutralizing antibody normally sufficient for cattle protection with a conventional vaccine are approximately one-tenth those shown in Table 2. Consequently, it is not yet possible to establish the efficacy of an FMD peptide vaccine on the comparative basis of its SNT value with that of a conventional vaccine. The first three animals that were challenged 21 days after reimmunization were completely protected. Antibody titers in these animals as measured by ELISA techniques increased after booster immunization. Currently, this synthetic peptide is the smallest agent in molecular size capable of inducing protection in cattle. Its synthetic nature will allow evaluation of its inherent activity in combination with various adjuvants, free of any bacterially derived contaminants.

REFERENCES AND NOTES

1. G. N. Mowat, A. J. Garland, R. E. Spier, *Vet. Rec.* **102**, 190 (1978).
2. D. G. Kleid *et al.*, *Science* **214**, 1125 (1981).
3. E. Pfaff, M. Mussgay, H. O. Bohm, G. E. Schulz, H. Scaller, *EMBO J.* **1**, 869 (1982).
4. J. Laporte, J. Grosclaude, J. Wantyghem, S. Bernard, P. Rouze, *C. R. Acad. Sci.* **276**, 3399 (1973).
5. H. L. Bachrach, D. M. Moore, P. D. McKercher, J. Polatnick, *J. Immunol.* **115**, 1636 (1975).
6. H. L. Bachrach, D. O. Morgan, P. D. McKercher, D. M. Moore, B. H. Robertson, *Vet. Microbiol.* **7**, 85 (1982).
7. D. G. Kleid *et al.*, in *Microbiology–1985*, L. Leive, Ed. (American Society for Microbiology, Washington, DC, 1985), p. 405.
8. K. Strohmaier, R. Franze, K.-H. Adam, *J. Gen. Virol.* **59**, 295 (1982).
9. J. L. Bittle *et al.*, *Nature (London)* **298**, 30 (1982).
10. T. R. Doel and A. D. Murdin, *Proceedings of the Fourth Meeting of the European Group on Molecular Biology of Picornaviruses*, Seillac, France, 1 to 7 September 1985.
11. N. R. Parry *et al.* [in *Vaccines 85*, R. A. Lerner, R. M. Chanock, F. Brown, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1985), p. 211] have independently reported a possible advantage of a similar concept. A different peptide, formed by linking both antigenic sites, was effective in competitive inhibition of a neutralizing monoclonal antibody binding to virus.
12. R. B. Merrifield, L. D. Vizioli, H. G. Boman, *Biochemistry* **21**, 5020 (1982).
13. J. Carlsson, H. Drevin, R. Axen, *Biochem. J.* **173**, 723 (1978).
14. S. M. Golding, R. S. Hedger, P. Talbot, J. Watson, *Res. Vet. Sci.* **40**, 142 (1976).
15. M. M. Hardy and D. M. Moore, *J. Gen. Virol.* **62**, 287 (1982).

3 September 1985; accepted 28 January 1986

A Mutation in the R Body–Coding Sequence Destroys Expression of the Killer Trait in *P. tetraurelia*

JUDITH A. DILTS AND ROBERT L. QUACKENBUSH

This report describes a mutant strain of *Caedibacter taeniospiralis* 169 that does not produce refractile (R) bodies or kill sensitive paramecia, but still renders its host resistant to killing by wild-type strains of *Caedibacter taeniospiralis*. The mutation is due to insertion of a 7.5-kilobase, transposon-like element into the R body–coding region of the plasmid pKAP169. The results provide strong evidence that R body synthesis is required for expression of the killer trait.

FORMERLY THE KILLER TRAITS OF *Paramecium* were considered classic examples of cytoplasmic inheritance. It is now known that these traits are due to the presence of bacterial cytoplasmic endosymbionts rather than naked genes (*1*). The most notable group of bacterial endosymbionts that occur in paramecia is the genus *Caedibacter* [commonly known as kappa particles (*2*)]. All caedibacteria are obligate endosymbionts of paramecia and typically exhibit two morphological forms. Most members of any population of caedibacteria do not have any distinguishable internal structures and are referred to as nonbright particles. The remaining individuals, called bright particles, possess a large, hollow, cylindrical inclusion body (approximately 0.5 μm long by 0.5 μm in diameter) known as a refractile body or R body. Only caedibacteria carrying R bodies are toxic to sensitive strains of paramecia (*3*). However, it is unlikely that R bodies themselves constitute the toxin, since purified R bodies from most strains of *Caedibacter* do not elicit toxic effects when ingested by sensitive paramecia (*1*) and since strains of *Escherichia coli* carrying cloned R body–coding sequences from *Caedibacter taeniospiralis* produce R bodies but do not elicit toxic effects when ingested by sensitive paramecia (*4*).

The question that arises is whether R body synthesis, which is a plasmid-determined trait in *C. taeniospiralis*, is required for expression of a killer trait, or whether the observed association between these traits in *Caedibacter* is merely coincidental. The available evidence suggests that R body synthesis and expression of toxigenicity are associated, but does not indicate whether one is required for the other. Widmayer (*5*) reported a mutant of *C. taeniospiralis* 51 (51m43) that had greatly reduced abilities to synthesize R bodies and kill sensitive paramecia but still retained the ability to confer resistance upon its host. Dilts (*6*) reported that plasmids carried by *C. taeniospiralis* 51m43 appear to exist as concatemers, whereas they occur as monomers in the wild type. In addition, we have observed that the amount of plasmid DNA present in a population of *C. taeniospiralis* 51m43 is much less than one would expect from a population of *C. taeniospiralis* of a similar size. Thus we believe that the mutation that occurred in *C. taeniospiralis* 51m43 probably affected plasmid replication, which in turn may have affected R body and toxin synthesis. Unfortunately, *C. taeniospiralis* 51m43 apparently no longer exists. Thus the genetic basis for the mutant phenotypes in *C. taeniospiralis* 51m43 cannot be determined. In this report we describe a mutant of *C. taeniospiralis* 169 that has spontaneously lost the abilities to synthesize R bodies and mediate expression of the killer trait due to insertion of a transposon-like element into the DNA sequence required for R body synthesis.

The mutant strain of *C. taeniospiralis* 169 was discovered during routine examination of subcultures of *Paramecium tetraurelia*

Table 1. Results of tests for killing activity and resistance to killing. Cultures of paramecia were mixed together and observed 6 and 24 hours later to determine whether any characteristic prelethal effects were exhibited by individuals in the mixed cultures (*5*). Abbreviations: −, no killing activity observed; +, killing activity observed; K, killer; R, resistant to killing; NK, nonkiller; and S, sensitive to killing.

Paramecium strain	*Paramecium* strain					Trait
	51K	169	169-1	51S	152	
51K	−	−	−	+	+	K, R
169		−	−	+	+	K, R
169-1			−	−	−	NK, R
51S				−	−	NK, S
152					−	NK, S

J. A. Dilts, Biology Department, William Jewell College, Liberty, MO 64068.
R. L. Quackenbush, Department of Microbiology, University of South Dakota School of Medicine, Vermillion, SD 57069.

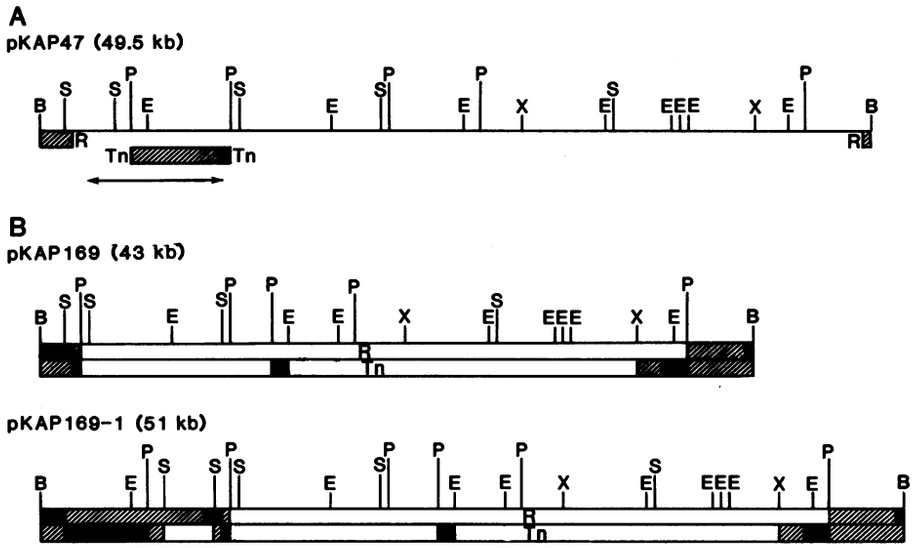
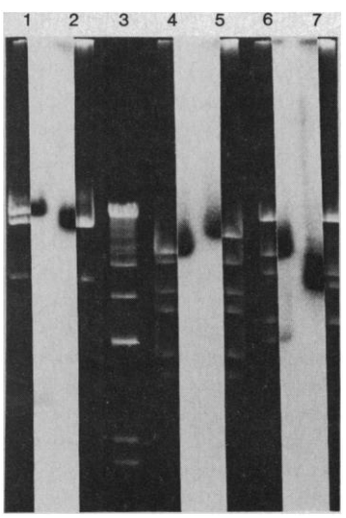


Fig. 1. Linearized maps of plasmids showing (A) sources of DNA probes obtained from derivatives of pKAP47 and (B) results of hybridization reactions of the probes with pKAP169 and pKAP169-1. (A) Restriction map of pKAP47 delineating sources of DNA probes used in hybridizations with Southern blots. The shaded area bounded by the letter R indicates the source of the sequences coding for R body synthesis. The shaded and solid areas bounded by Tn indicate the source of the sequence representing the right half of the 7.5-kb transposon-like element; the shaded region represents transposon sequence and the short solid region represents flanking sequence that is not part of the transposon. The arrow indicates the full extent of the 7.5-kb transposon sequence. (B) Restriction maps of pKAP169 and pKAP169-1. Sequences that were predicted to have homology with DNA probes obtained from derivatives of pKAP47 are indicated by solid areas. The row labeled "R" represents hybridization results obtained with R body–coding sequence as probe (Fig. 2) and the row labeled "Tn" represents hybridization results obtained with the transposon sequence probe (Fig. 3). Shaded and solid regions together indicate sequences observed to hybridize to DNA probes. Abbreviations: B, Bam HI; E, Eco RI; P, Pst I; S, Sst I; and X, Xho I.



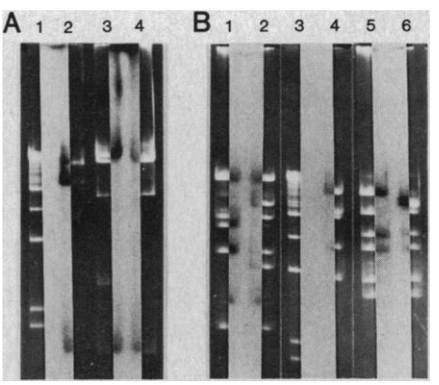


Fig. 2 (left). Hybridization of R body–coding DNA probe with pKAP169 and pKAP169-1. Probe DNA was a 2000-bp Cla I–Hae III fragment obtained from pBQ65, a recombinant plasmid derived from pKAP47 that allows R body synthesis to be expressed in *Escherichia coli* (*9*). Except for a maximum of approximately 300 bp located at the end of the probe to left of the Bam HI site (Fig. 1A), the sequences in the probe represent the full extent of sequences required for expression of R body synthesis (*9*). DNA samples were digested by restriction endonuclease, subjected to electrophoresis on an agarose gel, transferred to Gene Screen membrane (New England Nuclear), and hybridized with ^{32}P-labeled DNA probe (*10, 11*). The figure is a composite photograph of an ethidium bromide–stained agarose gel (dark background) and an autoradiograph of the Southern blot of that gel after hybridization with DNA probe. Lanes 1 and 2, Sst I digests of pKAP169-1 and pKAP169, respectively; lane 3, Hind III digest of λ*c*I857 DNA (fragment sizes are 23, 9.6, 6.7, 4.4, 2.3, 2.0, and 0.5 kb); lanes 4 and 5, Eco RI digests of pKAP169-1 and pKAP169, respectively; and lanes 6 and 7, Pst I digests of pKAP169-1 and pKAP169, respectively. Fig. 3 (right). Hybridization of Tn sequence DNA probe with pKAP169 and pKAP169-1. Probe DNA was a 5.5-kb Pst I fragment obtained from pBQ54, a recombinant plasmid derived from pKAP47 (*4*). (A) Lane 1, Hind III digest of λ*c*I857 DNA (see legend to Fig. 2 for fragment sizes); lanes 2, 3, and 4, Sst I digests of pKAP298, pKAP169-1, and pKAP169. (B) Lanes 1 and 2, Pst I digests of pKAP169 and pKAP169-1, respectively; lane 3, Hind III digest of λ*c*I857 DNA; and lanes 4, 5, and 6, Eco RI digests of pKAP298, pKAP169, and pKAP169-1, respectively.

169 derived from single-cell isolates. Observation by phase-contrast light microscopy of squashes of the ciliate host revealed nonbright forms of the endosymbiont; however, no R body–containing bright forms were observed. Examination of mutant endosymbionts purified from an 80-liter culture of host paramecia again failed to reveal any R body–containing individuals. The mutant endosymbiont and its ciliate host were both assigned the strain number 169-1.

Since bright forms of *Caedibacter* have been shown to be the toxic elements responsible for the killer trait (*3*), killing tests (*7*) were conducted to determine whether *P. tetraurelia* 169-1 had the killer trait. *Paramecium tetraurelia* 51S and *P. triaurelia* 152, which do not carry any endosymbionts, were used as sensitive indicator strains. *Paramecium tetraurelia* strains 51K and 169, both of which carry bright and nonbright forms of *C. taeniospiralis*, were used as killer controls. All strains used in the killing tests were examined microscopically for the presence of endosymbionts and R bodies. Only strains 169 and 51K contained both bright and nonbright forms. The results of the killing tests (Table 1) demonstrated that *P. tetraurelia* 169-1 differs from killer and sensitive indicator strains *Paramecium tetraurelia* 169-1 did not exhibit a killer trait but did show resistance against killing by the killer controls.

R body synthesis in *C. taeniospiralis* is a plasmid-mediated trait (*4*). The R body–coding plasmids that occur in all strains of *C. taeniospiralis* are highly conserved. The only differences detected among these plasmids are due to variations in molecular size, attributable to the presence or absence of three types of transposon-like DNA sequences (*8*). To determine the cause of the mutation at the molecular level, plasmids from strains 169 and 169-1 of *C. taeniospiralis* were purified and physically mapped by restriction endonuclease analysis (Fig. 1B). The restriction maps of these plasmids were found to be identical, except that a 7.5-kilobase (kb) sequence not present in pKAP169 has apparently been inserted in the sequence of 1500 base pairs (bp) between the Bam HI and Sst I sites at the left end of the restriction map of pKAP169 to form pKAP169-1 (Fig. 1B). This observation explains why strain 169-1 has lost the ability to synthesize R bodies, since DNA sequences required for the expression of R body synthesis include this 1500-bp sequence (*4, 9*). To confirm that the 7.5-kb inserted sequence does reside in the R body–coding region, Southern blots containing restriction endonuclease–treated pKAP169 and pKAP169-1 were hybridized to a radiolabeled DNA probe that included

the intact R body–coding sequence. The results (Figs. 1B and 2) confirm that the R body–coding region of pKAP169-1 is split by the 7.5-kb transposon-like sequence.

The 7.5-kb inserted sequence found in pKAP169-1 is similar to transposon-like sequences observed in the R body–coding plasmids pKAP47 and pKAP298 with respect to both size and distribution of restriction sites (*8*). To determine whether these sequences are related, Southern blots containing restriction endonuclease–digested pKAP169 and pKAP169-1 were hybridized to a radiolabeled DNA probe that included about two-thirds of a 7.5-kb element from pKAP47 (Fig. 1A). The results (Figs. 1B and 3) demonstrate that these sequences share a high degree of homology and strongly support the hypothesis that they are transposons. We previously reported that, in several strains of *C. taeniospiralis*, sequences having homology with the 7.5-kb transposon-like elements occur in other replicons within the endosymbiont genome, most likely in the chromosome (*8*). Thus pKAP169-1 probably resulted from transposition of a 7.5-kb transposon-like element residing in the *C. taeniospiralis* 169 chromosome into the R body–coding sequence of pKAP169.

We realize that the results presented here do not absolutely preclude the possibility that *C. taeniospiralis* 169-1 may be the result of two separate mutational events, one inactivating the R body–coding sequence and the other inactivating the toxin-coding sequence, the nature and location of which are unknown. However, the probability of both events occurring in the same cell is extremely low. In addition, our results are consistent with previous reports that killing activity is affected by the rate of R body production in *Caedibacter* (*3, 5*).

It is our conclusion that, although the genetic loci for R-body and toxin synthesis are probably separate, synthesis of intact R bodies is required for the toxin component to be active against sensitive paramecia. The evidence also suggests that (i) synthesis of intact R bodies is not required for resistance of the ciliate host to killing mediated by bright forms of *C. taeniospiralis* and (ii) the killer trait is an example of cytoplasmic inheritance with respect to both the eukaryotic host and the bacterial endosymbiont.

REFERENCES AND NOTES

1. J. R. Preer, Jr., L. B. Preer, A. Jurand, *Bacteriol. Rev.* **38**, 113 (1974).
2. R. L. Quackenbush, *J. Gen. Microbiol.* **108**, 181 (1978); *Int. J. Syst. Bacteriol.* **32**, 266 (1982).
3. J. A. Mueller, *J. Exp. Cell Res.* **30**, 492 (1963); J. E. Smith-Sonneborn and W. J. Van Wagtendonk, *ibid.* **33**, 50 (1964).
4. R. L. Quackenbush and J. A. Burbach, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 250 (1983).
5. D. J. Widmayer, *Genetics* **51**, 613 (1965).
6. J. A. Dilts, *J. Bacteriol.* **129**, 888 (1977).
7. T. M. Sonneborn, *Methods Cell Physiol.* **4**, 241 (1970).
8. R. L. Quackenbush, *Plasmid* **9**, 298 (1983); ———, J. A. Dilts, B. J. Cox, *J. Bacteriol.*, in press.
9. J. A. Kanabrocki, R. L. Quackenbush, F. R. Pond, in preparation.
10. E. M. Southern, *J. Mol. Biol.* **94**, 51 (1975).
11. P. W. J. Rigby, M. Dieckmann, C. Rhodes, P. Berg, *ibid.* **113**, 237 (1977).

12. We thank F. R. Pond for comments on the manuscript. Supported by NSF grant PCM-8113203 and by NIH grant GM36293.

11 October 1985; accepted 27 February 1986

Human Multidrug-Resistant Cell Lines: Increased *mdr*1 Expression Can Precede Gene Amplification

D.-W. SHEN, A. FOJO, J. E. CHIN, I. B. RONINSON, N. RICHERT, I. PASTAN, M. M. GOTTESMAN

The development of simultaneous resistance to multiple structurally unrelated drugs is a major impediment to cancer chemotherapy. Multidrug resistance in human KB carcinoma cells selected in colchicine, vinblastine, or Adriamycin is associated with amplification of specific DNA sequences (the multidrug resistance locus, *mdr*1). During colchicine selection resistance is initially accompanied by elevated expression of a 4.5-kilobase *mdr*1 messenger RNA (mRNA) without amplification of the corresponding genomic sequences. During selection for increased levels of resistance, expression of this mRNA is increased simultaneously with amplification of *mdr*1 DNA. Increased expression and amplification of *mdr*1 sequences were also found in multidrug-resistant sublines of human leukemia and ovarian carcinoma cells. These results suggest that increased expression of *mdr*1 mRNA is a common mechanism for multidrug resistance in human cells. Activation of the *mdr*1 gene by mutations or epigenetic changes may precede its amplification during the development of resistance.

SIMULTANEOUS RESISTANCE TO MULTIPLE cytotoxic drugs occurs frequently in tissue culture cells selected for resistance to a single agent and has been suggested as a mechanism of resistance to chemotherapy in human tumors. Multidrug-resistant cell lines have been isolated in tissue culture by multistep selection. Early steps occur with very low frequency in most cell lines unless the cells are treated with a mutagen; later steps occur more readily and cells can be rapidly adapted to relatively high levels of the selecting drug (*1*). Recently, we found that two DNA sequences, referred to as *mdr*1 and *mdr*2, are amplified in multidrug-resistant human KB carcinoma cell lines (*2*). These sequences were detected and cloned by hybridization with a homologous probe derived from the *mdr* gene, which is amplified in multidrug-resistant Chinese hamster cell lines (*3*). A human genomic clone, corresponding to one of the amplified *mdr* sequences and designated pMDR1, hybridizes to a messenger RNA (mRNA) of 4.5 kb that is expressed in the multidrug-resistant cell lines (*2*). We now examine expression of the *mdr*1 mRNA during the development of multidrug resistance in KB carcinoma cells, as well as in two other human multidrug-resistant cell lines of different origin. Since even low levels of cellular multidrug resistance may result in clinically refractory tumors, we were especially interested in analyzing *mdr*1 expression in the sublines with low (two- to sixfold) relative drug resistance.

The isolation and some properties of the human multidrug-resistant KB carcinoma cell lines have been described (*4, 5*). The KB cell lines used in this study, the manner of their selection, and their relative resistance to various drugs are shown in Table 1. The agents used in selecting different sublines in multiple steps were colchicine, Adriamycin, and vinblastine. In the first two steps of colchicine selection, clones could only be obtained if the cell populations were first mutagenized with ethyl methane sulfonate (EMS). Similarly, KB cell lines selected independently for resistance to Adriamycin or vinblastine (*4, 5*) were obtained only after mutagenesis with EMS in the first step. Subsequent selection, up to very high levels of resistance, was possible without mutagenesis, and occurred at high frequency (KB-A1, KB-V1). Also shown in Table 1 are the

D.-w. Shen and A. Fojo, Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20892.
J. E. Chin and I. B. Roninson, Center for Genetics, University of Illinois College of Medicine at Chicago, Chicago, IL 60612.
N. Richert, I. Pastan, M. M. Gottesman, Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20892.