# Casein Kinase IIα Transgene-Induced Murine Lymphoma: Relation to Theileriosis in Cattle

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Infection of cattle with the protozoan parasite *Theileria parva* results in a fatal lymphoproliferative syndrome that is associated with the overexpression of casein kinase II. The role of this enzyme in the pathogenesis of lymphoproliferative disorders was investigated by expressing the catalytic subunit in lymphocytes of transgenic mice. Adult transgenic mice displayed a stochastic propensity to develop lymphoma; co-expression of a c-*myc* transgene in addition to casein kinase II resulted in neonatal leukemia. Thus, the casein kinase II gene can serve as an oncogene, and its dysregulated expression is capable of transforming lymphocytes in a two-step pathway with c-*myc*.

Casein kinase II (CKII) is a heterotetrameric serine-threonine protein kinase that is composed of two catalytic subunits (α or α′) and two regulatory β subunits (*1*). The nucleotide and amino acid sequences of CKIIα are highly conserved throughout evolution: The human and rat proteins are identical for the first 328 of 389 amino acids; the mammalian and *Drosophila* proteins show 90% similarity (*2*). The unusual degree of sequence conservation and ubiquitous expression of CKII suggest that the enzyme may play a critical role in cell function. CKII has been postulated to regulate multiple pathways of cellular metabolism and gene expression (*3*), and it may itself be regulated during the cell cycle (*4*). Rapidly proliferating cells appear to show high levels of CKII activity; some human leukemias (*5*) and solid tumors (*6*) exhibit increased levels of CKII, suggesting a potential role in tumorigenesis.

A parasitic disease of cattle in eastern and central Africa has provided another example of an association between the dysregulated expression of CKII and a pathological process. Cattle infected with the tick-borne protozoan parasite *Theileria parva* develop a T cell lymphoproliferative disorder, termed theileriosis or East Coast fever, that is fatal within 3 to 4 weeks (*7*). Parasite-infected cells assume the appearance of lymphoblasts and are tumorigenic in nude mice. However, the timely treatment of infected animals with antiparasitic drugs can cure this pseudoleukemia; thus, the process is termed reversible lymphocyte transformation. Parasite-infected lymphoblastoid cells contain markedly increased amounts of CKII mRNA and protein relative to noninfected cells (*8*). To assess both the pathophysiological role of CKII in this process and its oncogenic potential, we have expressed the α catalytic subunit of CKII in the lymphoid compartment of transgenic mice.

With oligonucleotide primers based on the human CKIIα nucleotide sequence (*2*) and the use of the polymerase chain reaction (PCR), we amplified and cloned the mouse CKIIα coding sequence from first-strand spleen complementary DNA (cDNA) (*9*). Sequencing of the product revealed that the mouse and human coding regions are 94% identical at the nucleotide level; their predicted protein products share an amino acid identity of 97%. To confirm that the PCR product encoded a biologically active enzyme subunit, we expressed the mouse CKIIα cDNA in bacteria (*10*). The recombinant protein had a molecular mass of 42 kD (Fig. 1A), consistent with the predicted size of CKIIα, and it reacted on an immunoblot with antibodies to human CKIIα (Fig. 1B). Whereas most of the recombinant protein was insoluble, the nondenatured soluble fraction was able to phosphorylate a CKII substrate peptide (Fig. 1C).

The CKIIα cDNA was cloned into a vector (*11*) in which an immunoglobulin heavy chain promoter and enhancer direct expression to lymphocytes (Fig. 2A), and the construct was injected into the pronuclei of fertilized FVB/n mouse oocytes. Two transgenic founders were identified; they passed the gene to their offspring, creating the TG.CKA and TG.CKB lines (*12*). Ribonuclease protection analysis with a probe capable of distinguishing transgenic from endogenous CKIIα transcripts (Fig. 2A) revealed that the transgene was expressed in the lymphoid organs of animals of both lines, more abundantly in the thymus than in the spleen (Fig. 2B). Even in the thymus, the abundance of the transgene CKIIα mRNA was <10% of that of the endogenous CKIIα mRNA. Mice of these lines develop and breed normally, but pathological examination of organs from older mice suggests hyperplasia of the white pulp of the spleen.

CKIIα transgenic mice from both lines develop lymphoma in a stochastic manner. Of 139 mice examined for a median of 9 months, nine (6%) developed clinical and histological evidence of lymphoma. An additional 12 mice (9%) that did not show clinical evidence of lymphadenopathy or organomegaly were found dead; thus, the true incidence of lymphoma in this population lies between 6 and 15%. The earliest onset of disease was at 6.5 months. Most of

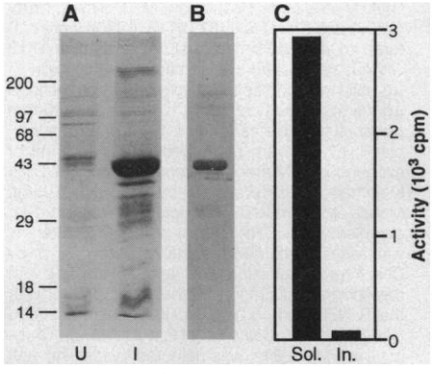


**Fig. 1.** Analysis of recombinant mouse CKIIα. (**A**) Polyacrylamide gel electrophoresis of 5 μl of transformed bacterial lysates revealed a band of ~42 kD in induced cells (I) that was not present in uninduced cells (U). The migration of molecular size standards (in kilodaltons) is shown on the left. (**B**) Immunoblot analysis of 0.2 μl of the induced bacterial lysate demonstrated that the recombinant material was cross-reactive with rabbit polyclonal antibodies to human CKIIα. (**C**) The catalytic activity of the soluble recombinant protein (Sol.) and the detergent-extracted insoluble bacterial pellet (In.) was determined by the incorporation of radioactivity from [γ-$^{33}$P]ATP into a CKII substrate peptide (*10*).

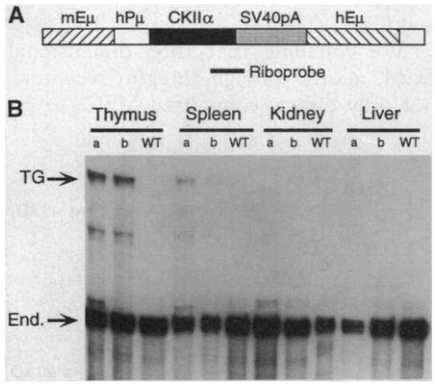


**Fig. 2.** Expression of a CKIIα transgene. (**A**) The CKIIα cDNA, subcloned into a vector with immunoglobulin promoter (hPμ) and enhancer (mEμ and hEμ) sequences as well as simian virus 40 intron and polyadenylate (SV40pA) sequences (*11*), was used to establish two lines of transgenic mice, TG.CKA and TG.CKB. (**B**) RNA prepared from tissues of wild-type (WT) and transgenic [TG.CKA (a) and TG.CKB (b)] mice was subjected to ribonuclease protection analysis with the antisense riboprobe indicated in (A). The endogenous CKIIα mRNA protects a band of 320 bases (End.), whereas the transgene mRNA protects a band of 420 bases (TG).

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the affected mice develop diffuse disease characterized by massive splenomegaly, hepatomegaly, thymic enlargement, and extensive lymphadenopathy, but generally without significant involvement of the peripheral blood. Histological examination of lymphoid organs revealed the effacement of normal architecture by monomorphic infiltrates of lymphoblastic cells with prominent nucleoli and frequent mitotic figures (Fig. 3A). Similar cells invade visceral organs including liver (Fig. 3B), kidneys, lung, bone marrow, and pericardium. Three of the mice developed more localized thymomas with the same histology; one mouse had a cutaneous lymphoma and thymoma with an atypical epithelioid histology.

We characterized the lineage of these tumors by immunofluorescent flow cytometry (*13*). Analyses of malignant cells dispersed from tissues of affected animals showed that they generally exhibit the cell surface phenotype of immature cortical thymocytes (Fig. 4, A and B). No B cell lymphomas have been detected. To assess the clonality of this lymphoproliferative disease, we looked for rearrangement of the T cell receptor Jβ gene. The wild-type and premalignant transgenic animals showed only the germline band in DNA prepared from lymphoid and nonlymphoid organs (Fig. 5), indicating that the T cells in those tissues had no single predominant rearrangement. In contrast, DNA from the T cell lymphomas showed novel bands, indicative of clonal rearrangements. The immunoglobulin μ locus usually retained the germline configuration, as would be expected for T lineage tumors (Fig. 5). The abnormal T cells were uniformly tumorigenic on transplantation into syngeneic FVB/n hosts, confirming their malignant nature.

These results demonstrate that dysregulated expression of CKIIα within lymphocytes can result stochastically in lymphoma. To investigate the possibility that the process of transformation might involve the further up-regulation of CKII, we assayed CKII in tumors derived from the transgenic mice. Ribonuclease protection analysis revealed an increase in transgenic CKIIα mRNA to 20% of the amount of endogenous CKIIα mRNA, consistent with either a modest up-regulation of transgenic mRNA or the enrichment of transgene-expressing cells in tumors. In concordance with the ribonuclease protection data, no significant increase in total CKIIα antigen, as assessed by immunoblot analysis, or in CKII enzymatic activity was observed in tumors from five different mice. Conversely, the tumors still required CKII, as demonstrated by the effect of antisense oligonucleotides to CKII on the growth of CKIIα tumor cell lines (*14*). The antisense oligonucleotides significantly inhibited the in-

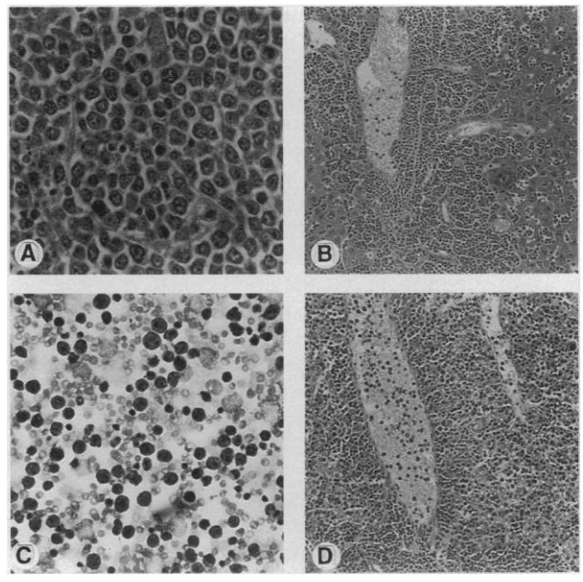


**Fig. 3.** Histology of the lymphoproliferative disease in CKIIα transgenic mice and in bi-transgenic CKIIα × c-*myc* offspring. (**A**) A lymph node from an adult CKIIα transgenic mouse that developed lymphoma shows the typical infiltrate of large lymphoblasts with prominent nucleoli and frequent mitotic figures. (**B**) Similar cells invaded visceral organs such as the liver, particularly in perivascular locations, but minimal host reaction or necrosis was apparent. Leukocytosis was not pronounced. (**C**) The peripheral blood of TG.CKA × TG.EB (c-*myc*) bi-transgenic offspring revealed that the disease presents as an acute lymphocytic leukemia at birth, with white blood cell counts of >400,000/mm$^3$. (**D**) The livers of these neonatal bi-transgenic mice were also extensively invaded, with marked hepatocellular damage apparent.

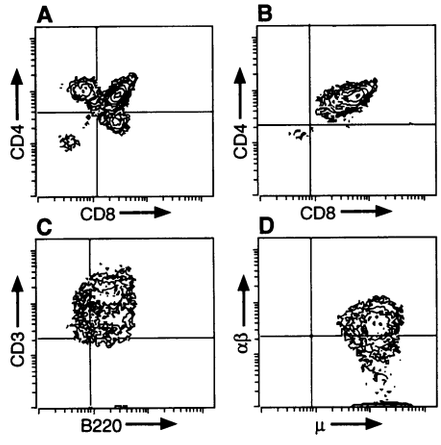


**Fig. 4.** Two-color flow cytometry analyses of the expression of lymphoid cell surface markers (*13*). (**A**) Normal thymus from a wild-type control mouse stained with antibodies to CD4 and CD8. About 4% of the cells were unstained, double-negative pro-thymocytes (lower left quandrant), 68% were more mature, double-positive thymocytes (upper right quandrant), and 18 and 10% were mature CD4$^+$ or CD8$^+$, single-positive T cells, respectively (upper left and lower right, respectively). (**B**) Thymus from a representative CKIIα transgenic mouse with lymphoma. The thymus was populated almost entirely with malignant cells bearing both CD4 and CD8. A few unstained cells, which may have been residual pro-thymocytes or non–T cells, were apparent, but no single-positive mature thymocytes were detected. The tumor cells also expressed T200, Thy1, CD2, CD3, and the αβ T cell receptor. (**C** and **D**) Analysis of a tumor cell line established from the bi-transgenic progeny of a TG.CKB (CKIIα) × TG.EB (c-*myc*) mating that developed lymphoma at <6 weeks of age. B220 and CD3 were co-expressed on all cells (C). These cells also expressed surface immunoglobulin μ chains and the αβ T cell receptor complex simultaneously (D), as well as κ light chains and low levels of CD4. Although the cells expressed Fc$^c$γRII receptors, staining with antibodies to B220, μ, κ, CD3, and the T cell receptor was not affected by preincubation with the Fc$^c$γRII blocking antibody 2.4G2 (*22*). The original tumor expressed low levels of B220 and CD3, but no other markers that were examined.

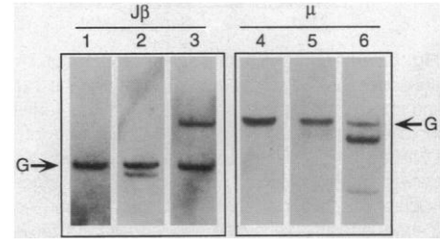


**Fig. 5.** Analysis of T cell receptor and immunoglobulin gene rearrangements in tumors. DNA prepared from tumors and wild-type control tissue was digested and subjected to Southern blot analysis. T cell receptor Jβ chain rearrangements (lanes 1 to 3) were detected with a probe (*23*) that identifies a single band in the germline configuration of genomic DNA digested with Hind III. Immunoglobulin μ heavy chain rearrangements (lanes 4 to 6) were detected with a probe (*24*) that identifies a single band in the germline configuration of genomic DNA digested with Eco RI. Lane 1, transgenic tail DNA illustrating the germline Jβ band (G). Lane 2, an example of a CKIIα tumor whose DNA contained both the germline band and a prominent clonally rearranged Jβ band. Lane 3, DNA from a CKIIα × c-*myc* bi-transgenic tumor. Lane 4, tail DNA showing the germline μ band. Lane 5, the germline μ configuration is preserved in most CKIIα tumors. Lane 6, the same tumor DNA as in lane 3 showed μ rearrangements in addition to the Jβ rearrangement.

corporation of [$^3$H]thymidine into DNA, whereas the corresponding sense oligonucleotides or mutated antisense oligonucleotides had no significant effect (Fig. 6).

Thus, although CKII expression is required for ongoing proliferation of the tumor cells, the mechanism of transformation in the transgenic mice does not involve further up-regulation of CKII. Transformation, therefore, must depend on the occurrence of one or more somatic events, such as the activation of other oncogenes or the deletion of anti-oncogenes. One candidate for a collaborating oncogene is c-*myc*, which has a demonstrated oncogenic role in lymphoma (*11*, *15*), is able to synergize with the serine-threonine protein kinase–encoding oncogenes *pim-1* (*16*) and *raf* (*17*), and may itself be regulated by CKII (*18*). To test whether c-*myc* might be capable of collaborating with CKII in lymphomagenesis, we mated together CKIIα and c-*myc* transgenic mice. The c-*myc* transgenic line TG.EB (*11*) has a human c-*myc* transgene that is also under transcriptional control of the immunoglobulin promoter and enhancers. TG.EB mice develop pre–B cell and, occasionally, T cell lymphomas at 4 to 9 months of age.

The co-expression of both of these transgenes resulted in a marked increase in the rate of onset of fatal lymphoproliferative disease. Bi-transgenic offspring resulting from the mating of heterozygous TG.CKA and TG.EB mice die by postnatal day 3, whereas their mono-transgenic littermates are healthy. Peripheral blood smears of 2- to 3-day-old bi-transgenic mice revealed acute lymphocytic leukemia, with white blood counts in excess of 400,000/mm$^3$ (Fig. 3C). Histological sections showed that the livers and spleens are full of lymphoblasts and revealed extensive hepatic necrosis (Fig. 3D). Bi-transgenic offspring of TG.CKB mice and TG.EB mice also exhibit the accelerated onset of disease, although some survive to adulthood.

Not only is co-expression of these two oncogenes rapidly transforming, but it can result in marked disruption of the regulation of lymphocyte gene expression. For example, one bi-transgenic TG.CKB × TG.EB tumor appeared to have a mixed lineage, with low-level surface expression of both B220 and CD3. Analysis of cells from this tumor in culture revealed that B220 and CD3 were strongly co-expressed on all cells (Fig. 4C), as were both surface immunoglobulin M (μ and κ chains) and the T cell receptor αβ complex (Fig. 4D). The T cell coreceptor CD4 was also expressed at low levels. The co-expression of two different receptor complexes was substantiated at the molecular level by analysis of tumor genomic DNA, which showed rearrangements in both the immunoglobulin and T cell receptor loci (Fig. 5). Thus, the coordinated action of the CKIIα and c-*myc* transgenes can result in the expression of elements of two normally exclusive programs of lymphoid differentiation.

The genes that encode the subunits of human CKII are located on chromosomes 20p13 (α), 16p13.3-13.2 (α′), and 6p21.1 (β) (*19*). Rearrangements of chromosome 20p13 have been detected in both acute and chronic lymphocytic leukemia and non-Hodgkin's lymphoma as well as acute myelogenous leukemia (*20*). Chromosome 16p13 has also been shown to be affected in some individuals with acute leukemia (*20*). Pulmonary hamartomas have been identified that have rearrangements of chromosome 6p21 (*21*). It will be important to investigate the potential role of CKII, perhaps in cooperation with other oncogenes such as c-*myc*, in the pathogenesis of these and other human cancers.

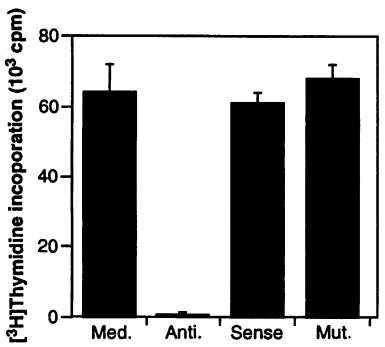


**Fig. 6.** Inhibition of tumor cell line proliferation by antisense CKII oligonucleotides (*14*). For the cell line shown, the antisense oligonucleotides (anti.) inhibited the incorporation of [$^3$H]thymidine into DNA by 98% relative to the incorporation observed with medium alone (med.). Control oligonucleotides, including the sense sequences (sense) and the antisense sequences with three nucleotide mutations (mut.) that have been shown to abrogate the antisense inhibition, had no significant effect. Inhibition of proliferation by antisense oligonucleotides was statistically significant in five of six tumor cell lines tested ($P < 0.05$) and was ≥90% in four of the six lines.

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9. First-strand cDNA was synthesized from total RNA that had been isolated [J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979)] from FVB/n mouse spleen, and the cDNA was then used as a template for PCR with oligonucleotide primers based on the human CKII nucleotide sequence (*2*) that had Bam HI recognition sites added at their 5′ ends. The sense oligonucleotide was 5′-AAAGGATCCATGTCGGGACCCGTGCCAA-3′ and the antisense oligonucleotide was 5′-AAAGGATCCTTACTGCTGAGCGCCAGC-3′. PCR was performed for 30 cycles of denaturation for 20 s at 96°C, annealing for 30 s at 56°C, and extension for 1.5 min at 75°C by Vent polymerase, which possesses proofreading exonuclease activity. The expected 1.2-kb product was gel-purified with GeneClean (Bio101), digested with Bam HI, cloned into pGEM-7zf$^+$ (Promega), and sequenced (U.S. Biochemical). The sequence has been filed with GenBank, accession number U17112.
10. The CKIIα cDNA was subcloned into an inducible vector for bacterial expression (pQE-8; Qiagen) and transformed into M15[pREP4] bacteria. Noninduced bacteria and bacteria induced with 2 mM isopropyl-β-D-thiogalactopyranoside were harvested and lysed by boiling in loading buffer for polyacrylamide gel electrophoresis, which was performed under reducing conditions. The separated proteins were transferred to an Immobilon-P nylon membrane (Millipore) and then subjected to immunoblotting with rabbit polyclonal antibodies to human CKIIα (Upstate Biochemicals), alkaline phosphatase–conjugated goat antibodies to rabbit immunoglobulin G, and nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Vector Laboratories, Burlingame CA). For assessment of enzymatic activity, induced bacteria were sonicated in phosphate-buffered saline, and the lysate was centrifuged to separate soluble from insoluble material. The catalytic activity of the soluble recombinant protein and the detergent-extracted insoluble bacterial pellet was assessed by incubation for 10 min at room temperature with 1 mM of the CKII substrate peptide RRREEETEEE (R, Arg; E, Glu; and T, Thr) [E. A. Kuenzel and E. G. Krebs, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 737 (1985)] and 400 nM [γ-$^{33}$P]ATP (adenosine triphosphate) (Dupont Biotechnology Systems) in 25 μl of 100 mM tris-HCl (pH 8.0), 100 mM NaCl, 50 mM KCl, 20 mM $MgCl_2$, and 100 μM sodium orthovanadate. The reaction was stopped with 25 μl of 10 mM ATP in 0.4 M HCl, and the mixture was applied to a P-81 filter disc (Whatman), which was washed extensively with 75 mM phosphoric acid before determination of the incorporated radioactivity. The CKIIα antigen and enzymatic activity in wild-type and transgenic tissues and in tumors were similarly determined. For these experiments, tissues were sonicated in the presence of protease inhibitors, and 50 μg of protein was subjected to immunoblotting and enzymatic assay as above.
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12. For the generation of transgenic lines, the CKIIα cDNA was subcloned into a vector, pIgTE/N, that was designed to direct expression to the lymphoid compartment (*11*). Plasmid sequences were digested and DNA was purified by electroelution from a gel fragment for injection into fertilized oocytes. Carriers of the transgene were identified by Southern (DNA) blotting with an [α-$^{32}$P]deoxycytidine triphosphate–labeled CKIIα cDNA probe and then bred to establish two founder lines, TG.CKA and TG.CKB [Institute of Laboratory Animal Resources nomenclature: TgN(EPmCKIIα)CKALed and TgN(EPmCKIIα)CKBLed, respectively]. CKII mRNA in tissues and tumors from these lines was assayed by ribonuclease protection [P. A. Krieg and D. A. Melton, *Methods Enzymol.* **155**, 397 (1987)], for which 7.5 μg of RNA was hybridized with the antisense [α-$^{32}$P]uridine triphosphate–labeled riboprobe indicated in Fig. 2A.
13. For flow cytometry, tissues were mechanically dispersed and the cells were washed in Hanks balanced salt solution, incubated for 30 min with fluorescently labeled antibodies, washed thoroughly, and analyzed on a CytoFluorograf II (Ortho Diagnostics). Antibodies to the following antigens were ob-

tained from the indicated sources: B220 (6B2), CD3 (2C11), T cell receptor αβ (H57), T cell receptor γδ (GL3), T200, and 8C5 from Pharmingen; Thy1.1 (M5/49) from Dupont Biotechnology Systems; CD4, CD5, and CD8 from Becton-Dickinson; μ and κ from Southern Biotechnology Associates; J11d, PgP-1 (or CD44), L-PAM, MEL-14, MAC-1, and F4/80 from American Type Culture Collection.
14. Tumor cells were passaged serially in syngeneic mice or in culture in RPMI 1640 supplemented with 10% fetal bovine serum, 4 mM glutamine, penicillin (50 U/ml), streptomycin (50 μg/ml), 50 μM 2-mercaptoethanol, and 1% interleukin-2–conditioned medium obtained from a transfected cell line [H. Karasuyama and F. Melchers, *Eur. J. Immunol.* **18**, 97 (1988)]. To assess the inhibitory effect of antisense CKII oligonucleotides on the growth of the tumor cells, we used a pair of oligonucleotides directed against CKIIα and CKIIβ transcripts that have been shown to inhibit the proliferation of fibroblasts induced by epidermal growth factor [R. Pepperkok, P. Lorenz, R. Jakobi, W. Ansorge, W. Pyerin, *Exp. Cell Res.* **197**, 245 (1991)]. Oligonucleotides (final concentration, 100 μg/ml) were added to $10^5$ cells plated in quadruplicate in 100 μl of medium prepared with heat-inactivated serum to improve oligonucleotide stability. After 20 hours of culture, [$^3$H]thymidine (1 μCi per well) was added, and after a further 4 hours, the cells were harvested and the incorporation of [$^3$H]thymidine into DNA (mean ± SD) was determined by scintillation counting.
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25. We thank S. Sansing for microinjection of the transgene construct; J. Campos-Torres for performing flow cytometry; J. Rush and the Biopolymers Facility for synthesizing oligonucleotides and peptides; and J. Walls and R. Cardiff of the Transgenic Mouse Pathology Laboratory at the University of California, Davis, School of Medicine, for preparation and interpretation of histological sections. Supported by the Howard Hughes Medical Institute and NIH award 1-K08-HL0286-01.

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# Selfish Behavior of Restriction-Modification Systems

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**Plasmids carrying gene pairs encoding type II DNA restriction endonucleases and their cognate modification enzymes were shown to have increased stability in *Escherichia coli*. The descendants of cells that had lost these genes appeared unable to modify a sufficient number of recognition sites in their chromosomes to protect them from lethal attack by the remaining restriction enzyme molecules. The capacity of these genes to act as a selfish symbiont is likely to have contributed to the evolution of restriction-modification gene pairs.**

Type II restriction endonucleases introduce double-strand breaks at specific short recognition sequences in duplex DNA provided that the sequences have not been modified by cognate methylases (*1*). Genes that encode cognate restriction-modification (rm) enzymes are normally tightly linked. The conventional hypothesis is that the rm gene pairs evolved under selection pressure to protect cells from infection by foreign (for example, viral) DNA. But it is not clear whether such a "cellular defense" hypothesis explains the extreme diversity and the exceptionally high specificity in their sequence recognition. This hypothesis cannot readily explain the evolution of the "rare cutter" restriction enzymes whose long recognition sequences (~8 base pairs) are unlikely to be present in many bacterial viruses. We present evidence for an alternative hypothesis that the evolution of some rm gene pairs has been driven by their behavior as "selfish" genetic units.

During an analysis of the role of DNA double-strand breaks in homologous recombination in *E. coli* (*2*), we observed that a plasmid carrying the rm genes of Pae R7 could not be readily displaced by a second plasmid devoid of the Pae R7 recognition sequence 5′-CTCGAG-3′. In the initial experiments, the resident $r^+m^+$ plasmid pIK137 [pPAORM3.8 in (*3*)] and the introduced plasmid pIK52 were incompatible, because both plasmids are driven by a ColE1 replicon. Plasmid pIK52 showed a much lower transformation efficiency when the *E. coli* host carried pIK137 as compared to pBR322, a plasmid without the $r^+m^+$ genes (Table 1). In addition, the majority of the colonies transformed with pIK52 DNA (42 of 52) were small, and all 24 of the transformants examined retained the resident $r^+m^+$ plasmid as well as the introduced plasmid (Fig. 1A). When the resident plasmid was an $r^-m^+$ version of pIK137 (pTN4), the transformation efficiency was not reduced, none of the 300 transformed colonies was small, and none of the 12 transformants examined retained a visible amount of the initially resident plasmid (Fig. 1B). These results suggested that restriction was responsible for the stable retention of the $r^+m^+$ plasmid.

When replication of the introduced and resident plasmids was driven from different compatible replicons, the presence of an $r^+$ gene as well as an $m^+$ gene in the resident plasmid did not influence the transformation efficiency (Table 1). Thus, the low transformation efficiency of pIK52 in the initial experiment was likely due to the resident pIK137 plasmid's enhanced stability, conferred by the rm genes.

To determine whether the $r^+m^+$ genes could stabilize a plasmid in the absence of a challenge by an incompatible plasmid, we inserted wild-type ($r^+m^+$) and mutant ($r^-m^+$) alleles of the Pae R7 rm genes into two different plasmid vectors, one (pBR322) driven by a ColE1 replicon, the other (pHSG415) driven by a pSC101ts replicon (*4*). The insertions into pBR322 were large, and the $r^-m^+$ version was less stable than pBR322. Nevertheless, the $r^+m^+$ version was found to be considerably more stable than even pBR322 itself (Fig. 2A). The r gene also had a pronounced stabilizing effect on pHSG415 (Fig. 2B). This pattern was reproduced with another type II rm gene pair, Eco RI (Fig. 2C).

On the basis of these results, we postulated the following mechanism to explain the plasmid stabilization. With successive generations, the descendants of a cell that has lost its $r^+m^+$ plasmid will contain fewer and fewer molecules of modification en-

**Table 1.** Resistance of a Pae R7 $r^+m^+$ plasmid to displacement. *Escherichia coli* strain JC8679 (*7*) carrying the indicated plasmids were transformed by electroporation (*2*) with 500 ng of pIK52 (*9*) or 0.5 ng of pIK134 (*10*). Transformants were selected on LB agar supplemented with kanamycin (50 μg/ml) or chloramphenicol (25 μg/ml), respectively.

| Resident plasmid | Transformation efficiency | |
|---|---|---|
| | pIK52 ($r^-m^-$)* (incompatible) | pIK134 ($r^-m^-$) (compatible) |
| pBR322 ($r^-m^-$) | 1 | 1 |
| pIK137 ($r^+m^+$) (*3*) | 0.11 | 2.3 |
| pTN4 ($r^-m^+$) (*8*) | 4.0 | 1.8 |
| None | 52 | 2.1 |

*Average of two experiments; differences between the values obtained were no greater than 10%.

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