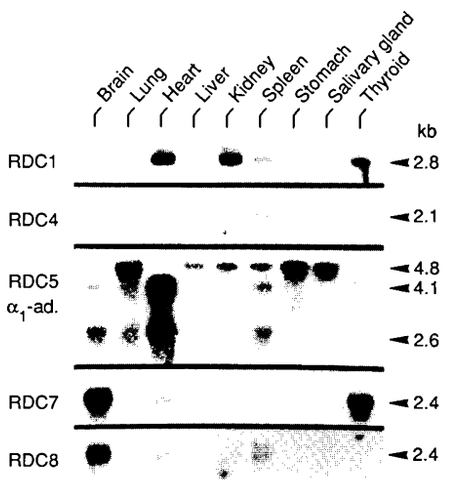

Fig. 3. Tissue-specific expression of four putative G protein–coupled receptors RDC1, RDC4, RDC7, RDC8, and the α_1-adrenergic receptor (RDC5). RNA blots were prepared with poly(A)$^+$ RNA extracted from nine different dog tissues (top) and probed with individual putative receptor sequences (left). The sizes were deduced from λ and φX174 DNA markers digested with Hind III and Hae III, respectively (right). The amount of RNA per lane was 5 μg (blots RDC1 and RDC8) and 20 μg (RDC4, RDC5, and RDC7). Selective precipitation in urea-LiCl medium (*24*) was used to isolate RNA from tissues of adult dogs. Poly(A)$^+$ RNA was extracted by oligo(dT) cellulose as described (*24*). Poly(A)$^+$ RNA were treated with glyoxal, fractionated on 1% agarose gels (*27*), and transferred to nylon membrane (Pall-biodyne). Hybridizations were performed with ^{32}P-labeled deoxyadenylate triphosphate probes (3×10^6 cpm/ml, 10^8 cpm/μg, random priming method). RDC1 and RDC8 probes were hybridized in the presence of 10% dextran sulfate.

minor cell populations present in the thyroid gland.

The approach described in the present study offers many advantages over homology cloning methods that are based on the screening of libraries with cross-hybridizing probes. The amplification provided by PCR together with the specificity achieved by two criteria of sequence similarity yield a low background compatible with the direct identification of clones by DNA sequencing. Careful selection of the primer sequences and degeneracies should make it possible to orientate the amplification process to specific subfamilies of genes. This will prove invaluable in cloning the many dozens of G protein–coupled receptors, the existence of which has been inferred from physiological and pharmacological evidence (*13–16*).

Note added in proof. After the present study was completed, a similar cloning approach was published (*17*).

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Murine MHC Polymorphism and T Cell Specificities

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The major histocompatibility complex (MHC) genes are polymorphic in mouse and man. The products of these genes are receptors for peptides, which while bound, are displayed to T lymphocytes. When bound peptides from antigens are recognized by T lymphocytes, an immune response is initiated against the antigens. This study assessed the relation of the polymorphic MHC molecules to their peptide specificity. The results indicate that although an individual of the species has a limited ability to recognize antigens, the species as a whole has broad reactivity. This rationalizes the extreme polymorphism observed.

A T HELPER LYMPHOCYTE, THROUGH its antigen-specific receptor, recognizes a peptide bound to a class II molecule, is activated, and stimulates an immune response to the antigen from which the peptide was derived (*1*). The primary structure of the NH_2-terminal polymorphic domain of the MHC molecule is essential for both T cell recognition and providing specificity for the binding of peptides (*2, 3*). Hence the immune responsiveness of the individual is determined in large part by the amino acid sequences of the class II molecule. The diversity of antigenic peptides recognized within the bacteriophage lambda repressor protein, cI (residues 1 to 102), was evaluated as a function of the polymorphisms of the class II molecules of the mouse species. We screened 13 different strains of mice; specific T cell hybrids generated from mice immunized with 1–102 were tested for with a panel of overlapping peptides spanning the entire protein (Fig. 1). Some strains (C57BL/6, B6.C-H-2^{bm12}, B10.D2, BALB/c, SM/J, P/J, SAF, and CLA) had only one target peptide, whereas others (B10.M, B10.RIII, B10S, B10.BR, and C3H.JK/Sn) had more than one. In the strains that had multiple targets, one was always immunodominant. The frequency of hybrids that recognized nondominant targets was usually about one-tenth that of hybrids recognizing the immunodominant target. Multiple MHC alleles can recognize the same target peptide. The class II molecules of different MHC haplotypes can display different T cell epitopes within the larger target peptide. For example, the part of 12–26 that is responsible for binding to I-A^d consists of residues 12 to 24, whereas the part of this same peptide which binds to I-E^k consists of residues 15 to 26 (*4–6*).

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We isolated some T cells that were not stimulated by peptides 10 to 15 residues in length, a length that is adequate for most T cells examined to date. For example, some T cells derived from B10.M mice required a peptide of 24 residues—namely, peptide 12–36—and were not stimulated by peptide 12–26, which stimulated T cells from several strains, (Table 1) or by peptide 21–36. Similarly, T cells from C3H.JK/SN mice required peptide 66–89 and were not stimulated by peptides 61–75, 69–82, or 73–88. Peptide 80–102 stimulated T cells of the P/J strain, which were not stimulated by shorter peptides, such as 80–94, 84–98, or 88–102. However, not all potential peptides in each case were tested. It is not clear what the basis is for this apparent requirement for longer peptides. It is unlikely that the class II molecule can accommodate long peptides in the presumed binding site; therefore, a particular conformation of the peptide may be induced that binds to the class II molecule.

The hybridomas derived from C57BL/6 (H-2^b) and B6.C-H-2^{bm12} (H-2^{bm12}) have the same target peptide, 73–88. We tested 23 individual hybridomas for their ability to recognize the peptide 73–88 in the context of either H-2^b or H-2^{bm12} antigen presenting cells (APC): H-2^{bm12} hybrids showed absolute specificity for H-2^{bm12} APC. Hybridomas derived from H-2^b can be divided into two groups. One group exhibits absolute specificity for H-2^b APC, whereas the other group can be triggered either by H-2^b or H-2^{bm12} APC. This observation supports the notion that a mutation (I-A^b to I-A^{bm12}) can lead to the acquisition of a determinant that may also function as a unique antigen-specific restriction site (*7, 8*).

We assumed above that the observed targeting is related to the binding specificity of the class II molecule. It is also possible that the processing of the antigen to generate peptides is dependent on the class II haplotype. Therefore, we immunized mice directly with peptides and observed the quantity of T cell reactivity to them (Fig. 2). Both BALB/c and C57BL/6 mice were immunized with peptide 12–26, 33–48 (negative for all strains tested), or 73–88. Seven days after immunization we examined the stimulation of lymph node–derived T cells in vitro. BALB/c mice responded only to peptide 12–26 as measured by induced proliferation; C57BL/6 mice responded to peptide 73–88 but not to 12–26 or 33–48. Thus the specificity observed within the T cell compartment was the same whether the whole protein or the individual peptides were used for immunization. In this system the differential processing of antigens in individuals with different haplotypes does not account for the specificity of T cell targeting. However, in other systems such as lysozyme (*9*) and myoglobin (*10*), immunization with peptides provides broader responses than seen with the intact protein. The reason for this is not clear. In addition, when we immunized BALB/c mice with a peptide mixture including 12–26, 33–48, 46–62, and 73–88, the T cells recognized only 12–26.

The target peptides are presumed to be class II–restricted and may provide T cell help for B cell responses. Therefore, T cells from mice immunized with a peptide that is a T cell target in the above experiments should be capable of inducing antibody to that peptide. We immunized both BALB/c and C57BL/6 mice with peptides 12–26, 46–62, and 73–88 and measured their primary and secondary antibody responses (Table 1). As expected, BALB/c mice produced

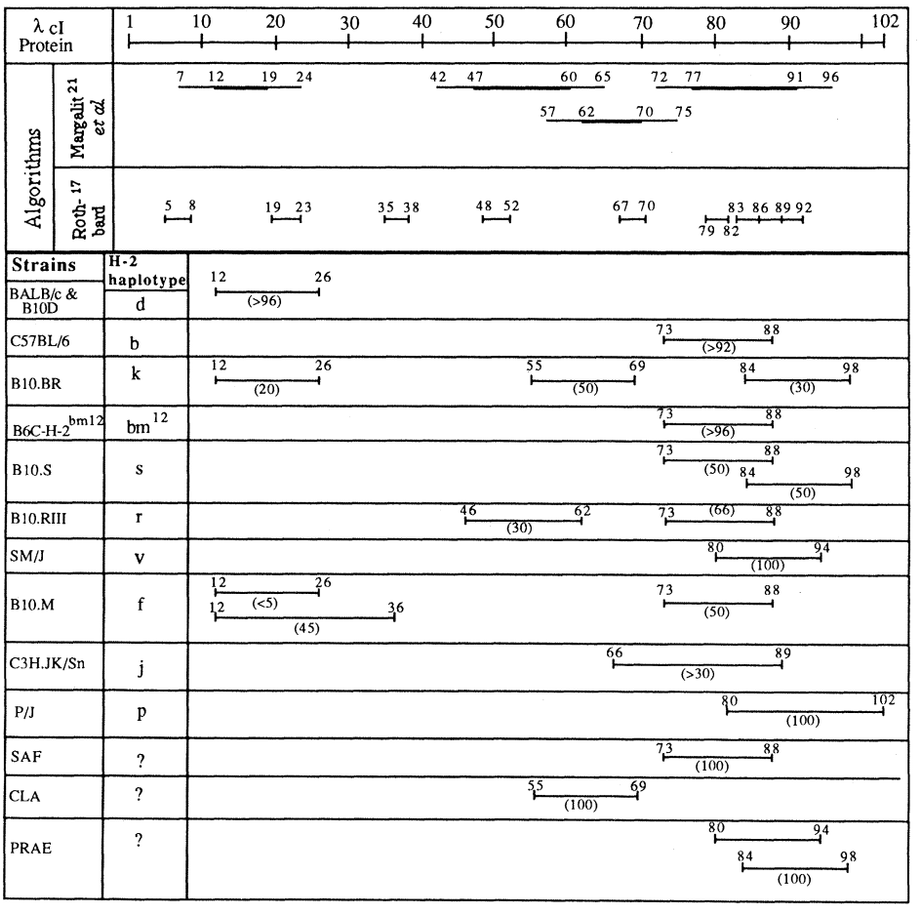

Fig. 1. T cell sites within cI (residues 1–102) in 13 mouse strains. At the top are the sites predicted by Margalit *et al.* (*23*) and Rothbard (*19*) algorithms as applied to residues 1–102. Thick lines on the Margalit (*23*) algorithm indicate the midpoints of 11 amino acid blocks, and thin lines indicate the extension of five residues on either side for starting and finishing points of the blocks. Numbers refer to the first and last residues in covalent structures. All of the peptides used in these experiments were made by the solid phase method of Merrifield (*26*) with an automated peptide synthesizer (Applied Biosystems 430A). Peptides used in this study for initial screening were 1–36, 12–36, 12–26, 33–48, 46–62, 66–89, and 80–102. For further refinement of T cell target region 66–84, peptides 61–75, 69–82, 73–88, and 55–69 were used. For refinement of the region 80–102, peptides 80–94, 84–98, and 88–102 were used. Mouse strains BALB/c (H-2^d), B10.D2 (H-2^d), B10.BR (H-2^k), C57BL/6 (H-2^b), B6.C-H-2^{bm12} (H-2^{bm12}), B10.M (H-2^f), SM/J (H-2^v), C3H.JK/Sn (H-2^j), and P/J (H-2^p) were obtained from the Jackson Laboratory (Bar Harbor, Maine). Generation of T cell hybrids after immunizing mice with 1–102 was as described (*27*). Hybrids were tested at a peptide concentration 10 μ*M*. Peptides shown routinely gave stimulation indices of least 20- to 100-fold when tested with T cell hybridomas (at least ten specific for each peptide) derived from each strain listed. Negative controls included several peptides from other portions of the protein, and they induced the same level of reactivity as control cultures without peptide added. The only exception were the SAF, CLA, and PRAE strains in which only visual analysis of growing CTLL indicator culture was used to score positive results. The number in parentheses under the lines refer to the approximate percentage of wells positive for the given peptide that were also positive for the immunogen 1–102. In some instances, clones could be not recovered from wells and therefore a certain assignment is not possible. For the entire study less than 2% of the clones could not be assigned to one of the peptides used. All wells of strain C3H.JK/Sn cells were weakly positive for 1–102, and the only recoverable clones were positive for 66–89. The exact minimal peptide for strain PRAE was not further determined.

Table 1. Antibody response to peptides from 1 to 102. BALB/c and C57BL/6 mice (three in each test group) were immunized intraperitoneally with 40 μg of peptide (12–26, 46–62, or 83–88) emulsified in complete Freund's adjuvant. Mice were bled before immunization (preimmune), 3 weeks after the first immunization (primary response), and again 1 week after the booster immunization of 40 μg of peptide emulsified in incomplete Freund's adjuvant (secondary response). Peptides were coupled to bovine serum albumin with 1-ethyl-3-(3 dimethylamino-propyl)carbodiimide HCL (ECDI). Briefly, peptide (5 mg) and 20 μl of ECDI (5*M*) were added to 100 μl of bovine serum albumin (10 μg/ml) and the mixture was placed on ice. After 1 hour, the reaction was quenched with sodium acetate (0.2*M*). Conjugated peptide (2 μg/ml) was adsorbed on Immulon-1 plates (Dynatech) and an enzyme-linked immunosorbent assay was carried out as described elsewhere (*28*). Values are means ± SD.

Strain	Peptide	Antibody response (μg/ml)		
		Preimmune	Primary	Secondary
BALB/c	12–26	5.3 ± 3.6	143 ± 99	518 ± 366
C57BL/6	12–26	3.2 ± 0	3.2 ± 0	5.3 ± 3.6
BALB/c	46–62	21 ± 10	28 ± 0	28 ± 0
C57BL/6	46–62	7.4 ± 3.6	21 ± 10	18.8 ± 13
BALB/c	73–88	18.8 ± 13	28 ± 0	21 ± 10
C57BL/6	73–88	18.8 ± 13	201 ± 99	201 ± 99

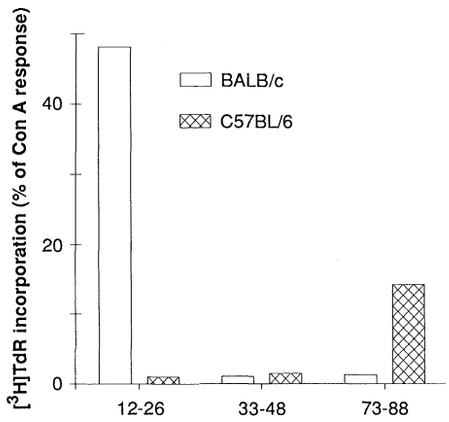

Fig. 2. The T cell response to peptides (12–26, 33–48, or 73–88) in BALB/c and C57BL/6 mice. Mice (four per group) were immunized in the footpads and the base of the tail with individual peptides (40 μg) emulsified in complete Freund's adjuvant (Difco). Seven days later, pooled draining lymph node cells (2.5×10^6 per milliliter) were stimulated in vitro with the same and unrelated peptides at concentrations of 5 to 40 μ*M*. Two days after in vitro culture, supernatant (50 μl) was collected and assayed for the presence of interleukin-2 (*27*). Maximum stimulation was obtained at 40 μ*M* concentration (data shown). Results are presented as the ratio of antigen-stimulated to concanavalin A (con A)–stimulated T cell proliferation (10 μg/ml). The con A response varied between 16×10^4 and 18×10^4 cpm. Background was 500 to 1000 cpm.

antibodies to 12–26 but not to 46–62 and 73–88, whereas C57BL/6 responded to 73–88 but not to 12–26 and 46–62. This observation demonstrated that the peptides 12–26 and 73–88 are immunodominant helper sites on 1–102 for BALB/c and C57BL/6, respectively. It remains to be shown in other systems studied, including the response to cytochrome c (*11*), myoglobin (*12, 13*), lysozyme (*14*), ovalbumin (*15*), influenza virus hemagglutinin (*16*), and staphylococcal nuclease (*17*) that immunodominant peptides determined by T cell reactivity actually provide "help" in their respective systems.

Many sequences can be recognized by the mouse species, but an individual of the species is restricted to a particular sequence or small set of sequences, the recognition of which is dependent on the MHC protein involved. Although not all strains used in this study are H-2 congenic, the B10 congenic series including haplotypes d, k, s, f, and r shows the general pattern observed with the 13 strains used. In the B10 congenic series, 70% of the lambda repressor molecule can be seen by the combination of B10 MHC-congenic strains. Two groups proposed algorithms to predict sites comprising T cell determinants (*18, 19*). The usefulness of both analyses have been demonstrated by their ability to identify previously unidentified T cell determinants (*20–22*). An analysis of amphipathic helices in 1–102 (Fig. 1) showed that the potential T cell recognition sites would be located within residues 8 to 25 and 43 to 97. Site 43–97 can be divided into three overlapping regions (43–66, 58–76, and 73–97) (*23*). Similarly, Rothbard and Taylor (*22*) showed that the pattern of a four– or five–amino acid motif is present within the sequence at sites 5–8, 19–23, 35–38, 48–52, 67–71, 79–82, 83–86, 86–89, and 89–92. We have seen T cells that recognize the predicted sites with two exceptions, sites 5–8 and 35–38. This may be due to either an insufficient number of haplotypes tested or to a flaw in the algorithm. For this test system, however, there is good agreement with both predictions. Target peptide 55–69 does not contain the motif described by Rothbard (*19*). The algorithms ignore the contribution of any particular allele of MHC molecule to sequence specificity for targeting, and thus for a given individual of an outbred population, the specific peptide recognized cannot be determined.

Strain B10.BR mice had T cells specific for three different sites (12–26, 55–69, and 84–98) in response to 1–102, whereas some strains (BALB/c, C57BL/6) responded only to one site. Thus, one individual may have a broad immune response to a protein that confers protection to a pathogen, whereas another individual may be only partially protected. Overall, the advantage of polymorphism is that it provides broad immune protection for the species as a whole (*24*).

Current ideas regarding antigen processing (generation of target peptides) suggest that proteins are subjected to proteolysis, and the resultant peptides are selected for binding by the MHC molecules of the individual. There is at this time no indication that the end product of processing in vivo is a peptide. Since virtually the entire antigen, at least in this case, can be seen by some member of the species, it does not seem reasonable that the resistance of the antigen to proteolysis would dictate possible peptide targets. Rather the processing event likely unfolds proteins or cleaves only at selective sites—for example, beta turns—and then the class II molecule binds to its preferred site. Subsequently proteolysis may trim the antigen to produce a more active T cell target.

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Interferon-α But Not AZT Suppresses HIV Expression in Chronically Infected Cell Lines

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Promonocytic (U1) and T lymphocytic (ACH-2) cell lines chronically infected with human immunodeficiency virus type 1 (HIV-1) constitutively express low levels of virus, but expression can be induced by phorbol esters and cytokines. Whereas ACH-2 cells produce infectious virions, U1 cells produce defective, noninfectious particles. Although 3′-azido-3′-deoxythimidine (AZT) prevented acute HIV infection of susceptible cells, it did not prevent the induction of HIV expression in the infected cell lines. In contrast, interferon alpha (IFN-α) inhibited the release of reverse transcriptase and viral antigens into the culture supernatant after phorbol ester stimulation of both cell lines. Further, IFN-α suppressed the production or release (or both) of whole HIV virions, but had no effect on the amount of cell-associated viral proteins. Also, after phorbol ester stimulation of ACH-2 cells, IFN-α reduced the number of infectious viral particles secreted into the culture supernatant, but had no effect on the infectivity of cell-associated virus. These findings lend support to the combined use of antiviral agents that have action at both the early (AZT) and the late (IFN-α) stages of HIV replication.

ACQUIRED IMMUNODEFICIENCY SYNdrome (AIDS) is characterized immunologically by depletion and functional impairment of the CD4-positive lymphocyte subset (*1*). Other cells of the immune system, such as B lymphocytes (*2*) and mononuclear phagocytes (*3*), are also functionally impaired, and can be targets of human immunodeficiency virus (HIV) infection (*4*). The onset of clinical symptoms often occurs years after initial infection with HIV (*5*), suggesting that a state of latency or permanent expression of virus exists for variable periods in the majority of patients. In support of this hypothesis, in vitro observations indicate that HIV can persist in both lymphocytic (*6, 7*) and monocytic (*8*) cells for long periods in a low replicative or latent form until activation signals trigger viral expression. Among the inductive signals associated with HIV replication in vitro are antigens (*9*), mitogens (*7, 10*), phorbol esters such as phorbol myristate acetate (PMA) (*11, 12*), and cytokines (*13, 14*), cellular transcription factors such as Sp1 (*15*) and NF-κB (*16*), and transfected genes of other viruses (*17*). Furthermore, monocytes and tissue macrophages are relatively resistant to the cytopathic effect of HIV (*3, 8*), suggesting that they might serve as circulating and tissue reservoirs of the virus. Therefore, the development of in vitro models reflecting the different stages of HIV infection (that is, acute compared to latent or chronic infection) in different cell types could be relevant to the design of broader and more effective antiviral strategies. In this regard, 3′-azido-3′-deoxythymidine (AZT) (*18*) and recombinant interferon alpha (IFN-α) (*19*) have shown antiretroviral activity and clinical efficacy in HIV-infected patients. In addition, IFN-α has shown efficacy in the treatment of AIDS-associated Kaposi's sarcoma (*19, 20*). Whereas AZT inhibits reverse transcription of viral RNA, an early phase of the viral cycle, and therefore prevents the integration of HIV provirus into the host genome (*21*), little is known about the mechanism of action of IFN-α on HIV in susceptible cells. Studies of murine retroviruses indicate that IFNs inhibit early events, such as transcription or translation of the viral genome during acute infection (*22*). However, when chronically infected cells were studied, IFNs predominantly affected the late stages of virion release from the plasma membrane (*22*).

Previously we developed two cell clones permanently infected with HIV-1. One clone (U1) was obtained by limiting dilution of cells surviving the acute infection of the promonocytic cell line U937. A second clone (ACH-2) was similarly derived from cells surviving the acute infection of the T lymphocytic cell line A3.01. The U1 and the ACH-2 cell clones have, respectively, two and one integrated copies of proviral DNA as determined by restriction enzyme analysis (*23*). In addition, both clones express low or undetectable constitutive levels of HIV.

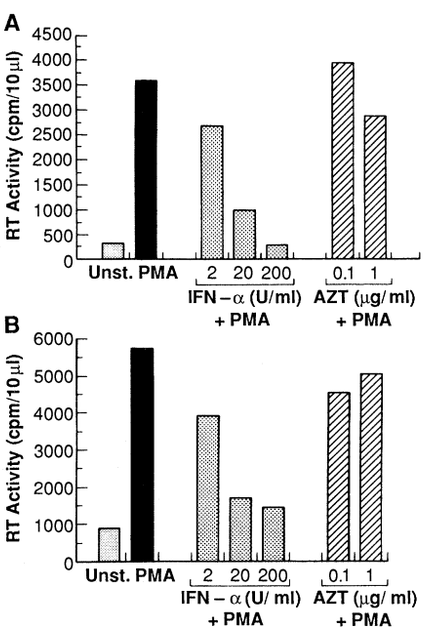

Fig. 1. Effect of IFN-α and AZT on HIV expression in chronically infected cells. Promonocytic U1 cells (**A**) and T lymphocytic ACH-2 T cells (**B**) were resuspended at 2.5×10^5 per milliliter in RPMI 1640 supplemented with 10% fetal calf serum. Cells were then stimulated with PMA ($10^{-7}M$) in the presence or absence of recombinant IFN-α (2 to 200 U/ml) or AZT (0.1 to 1 μg/ml) for 48 hours at 37°C in 7% CO_2. Unstimulated cells (unst.) were incubated under the same conditions. Culture supernatants were then harvested and tested for the presence of HIV RT activity (*33*). Briefly, 10 μl of supernatants were added in duplicate to 50 μl of a mixture containing polyadenylate, oligo(dT) (Pharmacia), $MgCl_2$, and [α^{32}P]-labeled deoxythymidinetriphosphate (dTTP) (Amersham) and incubated for 2 hours at 37°C. A sample (10 μl) of the mixture was then spotted on DE81 ion exchange chromatography paper (Whatman), air-dried, washed five times in 2× standard saline citrate buffer, and twice more in 95% ethanol. The paper was then dried, cut, and the radioactivity present was determined in a Beckman LS 7000 scintillation counter. Variability of replicate cultures was always less than 10%. The patterns represent more than ten independent experiments.

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