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# A Novel Nucleoprotein Complex at a Replication Origin

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**The viral protein p6, required for the protein-primed initiation of replication of *Bacillus subtilis* phage φ29, forms a nucleoprotein complex at the viral replication origins that shows novel features. Deoxyribonuclease I and hydroxyl radical footprinting data, as well as the induction of positive supercoiling, support a model in which a DNA right-handed superhelix tightly wraps around a multimeric p6 core. The interaction occurs through the DNA minor groove. The activity of p6 not only requires the formation of the complex but also its correct positioning, indicating that the other proteins involved in the initiation of replication recognize, at a precise position, either the p6 core or the DNA conformational change induced by p6.**

THE INITIATION OF REPLICATION OF circular (or topologically constrained) double-stranded DNA genomes is associated with the formation of a nucleoprotein complex at the replication origin that severely alters the DNA conformation and allows or stimulates the assembly of the replication machinery (*1*). Such complexes may also occur in circular genomes that replicate by a rolling-circle mechanism (*2*). The initiation of replication of linear double-stranded DNA genomes that have a terminal protein covalently linked at both 5′ ends has been studied mainly in adenovirus (*3*) and bacteriophage φ29 of *Bacillus subtilis* (*4*). In both systems there is evidence for the formation of nucleoprotein complexes at replication origins (*5–7*).

Adenovirus and φ29 initiate replication at both genome ends through the formation of a covalent linkage between the first deoxynucleoside monophosphate (dNMP) and a free molecule of the terminal protein, which is used as primer; the linkage is catalyzed by the viral DNA polymerase. Once the initiation reaction occurs, elongation proceeds, displacing the nontemplate strand until a genome-length strand is synthesized. In the case of φ29, in addition to the terminal protein and the DNA polymerase, the viral protein p6 is required for the phage DNA replication in vivo (*8*). Protein p6 stimulates both the initiation reaction and the transition to the elongation process in a φ29 DNA in vitro replication system (*9*).

Protein p6, which is very abundant in infected cells, interacts with double-stranded DNA (*7*) presumably as a dimer of 24 kD (*10*). By deoxyribonuclease I (DNase I) footprinting it was shown that protein p6 cooperatively forms a periodic DNA–multimeric protein complex at the φ29 replication origins (*7*) that extends along the terminal 200 to 300 bp. The major determinant of p6 binding seems to be the ability of these sequences to assume a well-defined conformation; this has been related to the ability of the DNA to bend (*11*). Binding of p6 to nonterminal φ29 DNA fragments has been observed, but it does not show the periodicity or salt-resistance of binding to terminal fragments (*7*). The formation of the nucleoprotein complex at the replication origins is required for the activity of protein p6 in the initiation of replication (*11, 12*).

The DNase I footprint pattern of p6 bound to the φ29 replication origins consists of strong hypersensitive bands periodically spaced every ~24 bp, the remaining positions being protected, except an unprotected or moderately hypersensitive site in the middle (*7*) (see also Fig. 1). Hydroxyl radical footprinting (*13*) was used to further study the interaction of protein p6 with the φ29 replication origins, since it allows one to determine the positions of the DNA backbone contacted by the protein with a

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high degree of resolution. Protein p6 protected regions of 3 to 4 nucleotides, regularly spaced every 12 to 13 nucleotides, and spanning all of the right-terminal fragment (Fig. 2). The same pattern was observed in the complementary strand, although shifted four nucleotides toward the 3′ end (Fig. 2). When the left replication origin was analyzed, the same pattern as in the right replication origin was observed and, furthermore, protected regions were located at the same positions relative to the genome end (*14*). The mean DNase I and hydroxyl radical footprint motif repeated along the ϕ29 replication origins are shown in Fig. 3A. Contact sites, defined as hydroxyl radical–protected regions at both DNA strands, shifted four nucleotides from each other, were flanked by strong DNase I–hypersensitive sites on alternating strands. The sites that were unprotected or moderately hypersensitive to DNase I were located in front of the strong ones, although displaced three nucleotides toward the 3′ side. Thus, the repeated motif, from a strong DNase I–hypersensitive site to the next one in the same DNA strand, contains two contact sites. The fact that the repeated unit is formed by two contact sites supports the conclusion that p6 binds as a dimer. When the above data are represented on a double-stranded DNA model (Fig. 3B), it becomes apparent that p6 interacts with DNA through the minor groove. In agreement with this, distamycin A, which binds in the minor groove, completely displaced p6 from DNA and inhibited the p6 stimulation of ϕ29 DNA replication (*14*). In addition, methylation protection experiments indicated that guanines were not protected at the N-7 position, which faces the major groove. However, some adenines and pyrimidines became hypersensitive to dimethylsulfate in the presence of p6 (*14*). This effect could be due to methylation of N-1 and N-3 positions of adenines and pyrimidines, respectively (*15*), that are usually hidden in the DNA double helix; this may reflect a DNA conformational change induced by p6 binding.

A major determinant of the DNase I cleavage rate is known to be the width of the minor groove (*16*). The DNase I–DNA cocrystal structure shows that DNase I is introduced deep into the DNA minor groove, bending DNA away from the protein (*17*). In agreement with this, there are several examples of DNase I digestion patterns of loops or bends in which DNase I preferentially cuts at positions where the minor groove is predicted to be opened (*18*). Therefore, a reasonable explanation for the DNase I strong hypersensitive sites induced by p6 binding to the ϕ29 replication origins could be that the DNA segments between the two p6 contact sites are forced into a bent configuration by protein-protein interactions. This would open up the outward-facing minor groove, optimizing the interaction with the DNase I. This hypothesis is supported by the fact that a strong DNase I–hypersensitive site in one strand and the medium hypersensitive site (or unprotected site) corresponding to the complementary strand are displaced by an average of three nucleotides toward the 3′ side (Fig. 3A). This spacing is the expected one for DNase I molecules interacting at a single DNA bend in the two possible orientations, each one cleaving a different strand (*17*). The preferential cutting of one DNA strand in each proposed DNA bend may reflect an irregular distortion of the DNA conformation or a steric hindrance by the protein.

It has been shown by one- and two-dimensional electrophoresis (*7, 14*) that protein p6 restrains positive supercoiling when bound to a circular DNA. If protein p6 binding imposes bends on DNA with a periodicity of 12 bp, which is larger than the double-helix pitch, the generation of a right-

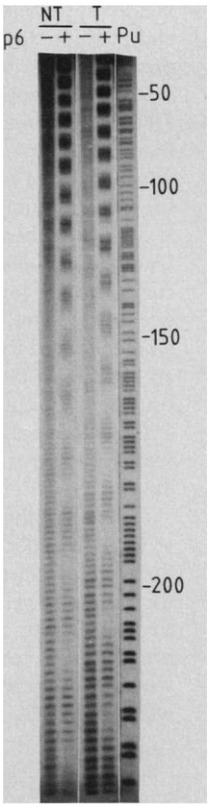


**Fig. 2.** Hydroxyl radical footprinting of protein p6 bound to the Hind III L right ϕ29 DNA terminal fragment (273 bp). Nontemplate (NT) and template (T) strands were labeled at the internal 3′ and 5′ end, respectively. When indicated, 4 μg of p6 was added. Pu, purine reaction of the nontemplate strand used as size marker (*24*). Numbers indicate positions from the genome end. For hydroxyl radical footprinting (*13*), purified p6 (*10*) was incubated with the appropriately labeled DNA fragment (~10 fmol) in a final volume of 20 μl containing 50 m*M* tris-HCl (*p*H 7.5), 10 m*M* $MgCl_2$, 20 m*M* $(NH_4)_2SO_4$, and 50 m*M* NaCl. Hydroxyl radical cutting was done at room temperature (2.5 min) by adding final concentrations of 0.3% $H_2O_2$, 0.1 m*M* ammonium iron (II) sulfate, 0.2 m*M* EDTA, and 1 m*M* ascorbic acid. Reactions were stopped as described (*13*). Samples were loaded on a 6% polyacrylamide–urea gel (*24*) after ethanol precipitation.

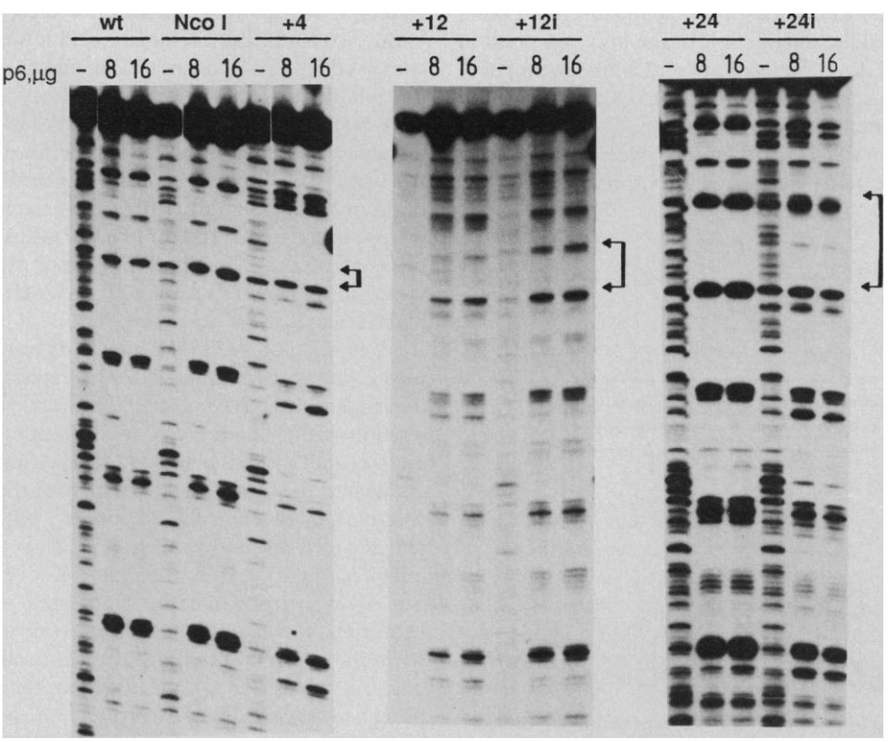


**Fig. 1.** DNase I footprinting of protein p6 bound to substitution and insertion mutants at the right ϕ29 replication origin. Construction of mutants was as described (*21*). DNA fragments were labeled in all cases at the internal 3′ end. The upper part of the gel corresponds to the genome end. Arrowed bars indicate the position of the different insertions. DNase I footprinting (*25*) was done as described in Fig. 2 except that samples were incubated for 2 min at 37°C in the presence of 20 ng of DNase I. Reactions were stopped as described (*25*).

handed superhelix would be expected. It is also possible that changes in DNA twist by protein p6 binding may also contribute to the induced topological change. In fact, DNA twist could be decreased up to less than 12 bp per turn provided that the positive writhe is large enough to account for the restraining of positive supercoiling. If twist were changed by 12 bp per turn, the binding of protein p6 would generate a flat curve, and the complex would restrain negative supercoiling. The extreme case in which all the induced topological change is due to an increase in twist is not favored, since it could only occur if p6 surrounded DNA, which would not explain the DNase I hypersensitivity as it is currently understood (*16–18*).

A model of the p6-DNA complex formed at the ϕ29 replication origins that explains both the DNase I and hydroxyl radical footprints, as well as the restraining of positive supercoiling is shown in Fig. 4. Protein-protein and protein-DNA interactions, would cooperatively form a p6 core around which the DNA is wrapped so as to form a right-handed superhelix. The p6 contact sites and DNase I–hypersensitive sites would face, respectively, inward and outward (Fig. 4). It has been previously reported that protein p6 does not recognize a specific sequence in the DNA through direct contacts with the bases; instead, its binding seems to be determined by the anisotropic flexibility of the DNA double helix (*11*). The proposed model makes it conceivable that the structural ability of the DNA to be bent every 12 bp can be a major determinant for the location of the protein in an ordered fashion. In addition, the binding of protein p6 to the DNA minor groove through short patches of the DNA backbone is consistent with the lack of recognition of a specific sequence in the DNA (*19*).

According to the hydroxyl radical footprinting experiments, the contact site closest to the genome end would be centered at positions 10 to 11, in the distal part of the terminal 12 bp that form the minimal replication origin (*20*). As a step to understand the functionality of the p6–DNA complex, we have studied whether the assembly of the nucleoprotein complex is enough for its activity, or whether the correct location of protein p6 relative to the genome ends is also required. To address this question, we constructed a set of DNA templates in which the phasing of the p6–DNA complex relative to the genome end is changed. Insertions were introduced between the minimal replication origin (*20*) and the main p6 binding determinants at the right ϕ29 replication origin, which had been mapped between positions 60 and 125 relative to the genome end (*11*). A single base pair substitution at position 39 from the right ϕ29 DNA end was generated to create a Nco I restriction enzyme target site (Nco I mutant). This restriction site was used to introduce insertions of 4 bp (+4 mutant), 12 bp, and 24 bp, the last two being almost identical to the ϕ29 DNA sequence that follows on the interior side of the insertion point in a direct orientation (+12 and +24 mutants) or in an inverted one (+12i and +24i mutants) (*21*).

The DNA fragments were tested for p6 binding by DNase I footprinting (Fig. 1).

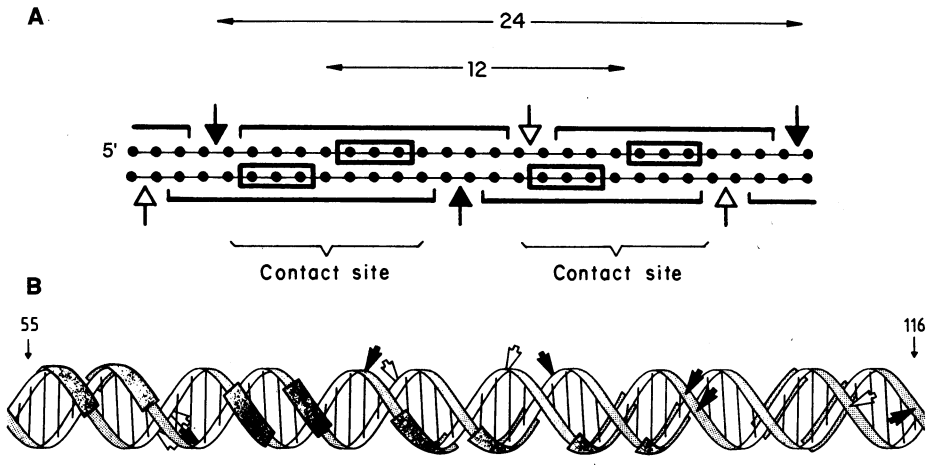


**Fig. 3.** (**A**) Mean DNase I and hydroxyl radical protein p6 footprint motif repeated along the ϕ29 replication origins. Nucleotide positions are represented by dots. The top strand is the nontemplate strand. Filled arrows indicate strong hypersensitive sites, and open arrows unprotected or moderately hypersensitive sites. Bars and boxes are DNase I– and hydroxyl radical–protected positions, respectively. Numbers are mean distances in base pairs. (**B**) Double-stranded DNA model with the DNase I and hydroxyl radical protein p6 footprint data corresponding to positions 55 to 116 from the right ϕ29 replication origin (7) (Figs. 1 and 2). Filled arrows, strong DNase I–hypersensitive sites; open arrows, unprotected or medium DNase I–hypersensitive sites; shaded boxes, hydroxyl radical–protected sites.

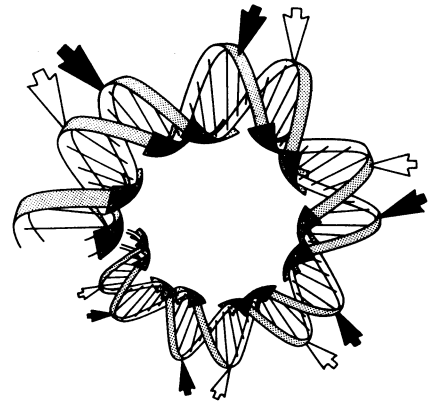

**Fig. 4.** Model of the nucleoprotein complex formed by protein p6 at the ϕ29 replication origins. Front view of about one superhelical turn. Superhelix curvature is arbitrary. A twist corresponding to a DNA of 10.5 bp per turn is considered. Filled arrows, strong DNase I–hypersensitive sites; open arrows, unprotected or medium DNase I–hypersensitive sites; shaded boxes, hydroxyl radical–protected sites.

As expected, the phasing of the complex revealed by the position of the DNase I–hypersensitive sites was mainly determined by sequences interior to the insertion point. Thus, the distance of the p6 molecules with respect to the genome end was increased 4 bp in the +4 mutant, and 12 bp in the +12 and +12i mutants, in comparison with the wild-type DNA, whereas it was not altered in the Nco I substitution mutant, and it was conserved in the +24 and +24i insertion mutants since the p6 binding periodicity is ~24 bp (Fig. 3A). In the +4 and +24i mutants an extra set of DNase I–hypersensitive bands was observed, probably as a result of p6 unspecific binding, since it selectively disappeared at about 100 m*M* NaCl (*14*). At this concentration, hypersensitive bands observed in fragments lacking ϕ29 DNA terminal sequences also disappear (*7*).

When the modified DNA fragments were used as templates in the ϕ29 in vitro replication system (*22*), p6 stimulated the initiation reaction with both the Nco I substitution mutant and the +24 and +24i insertion mutants to the same extent as that with the wild-type DNA (Fig. 5A). However, protein p6 failed to stimulate (Fig. 5A), or even inhibited initiation (*14*), when the +4, +12, and +12i insertion mutants were used as templates. Similar results were obtained when the ability of protein p6 to stimulate ongoing replication of the different templates was assayed (Fig. 5B). In this case, the replication reaction was performed under conditions in which the initiation reaction was not affected and the stimulatory effect of protein p6 was only due to the stimulation of the transition step between initiation and elongation (*9*). Therefore, although the pro

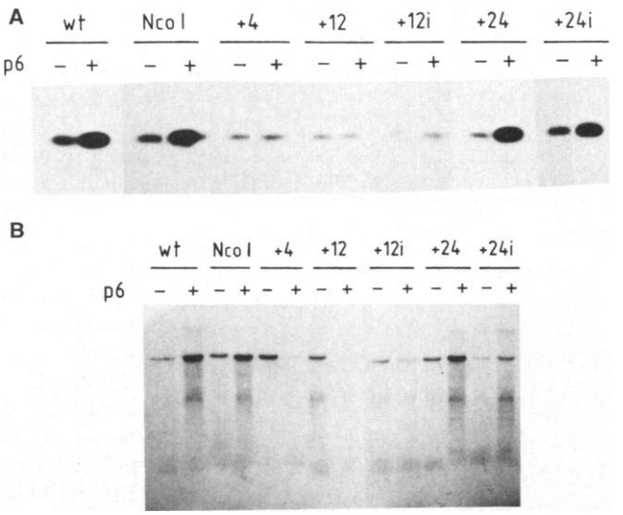


**Fig. 5.** Effect of p6 in the initiation and replication reactions with templates consisting of mutants of the right ϕ29 replication origin. (**A**) The incubation mixture for the initiation reaction contained, in 25 μl, 50 m*M* tris-HCl (*p*H 7.5), 10 m*M* $MgCl_2$, 20 m*M* $(NH_4)_2SO_4$, 1 m*M* dithiothreitol, 1 m*M* spermidine, 0.25 μ*M* [α-$^{32}$P]dATP (deoxyadenosine triphosphate) (2.5 μCi), 30 ng of purified terminal protein (*26*), 25 ng of purified ϕ29 DNA polymerase (*27*), 3 μg of purified protein p6 (*10*), when indicated, and ~6 fmol of the indicated DNA fragment. After incubation for 10 min at 30°C, the reaction was stopped by addition of EDTA to 10 m*M* and heating for 10 min at 68°C, and samples were treated with micrococcal nuclease. Unreacted [α-$^{32}$P]dATP was removed by filtration through a Sephadex G-50 spun column in the presence of 0.1% SDS and samples were finally subjected to SDS–polyacrylamide gel electrophoresis as described (*28*). The $^{32}$P-labeled terminal protein–deoxyadenosine monophosphate complex was detected by autoradiography of the dried gel. (**B**) For the DNA replication reaction, the incubation mixture was as described above except that it contained 70 m*M* NaCl and 20 μ*M* [α-$^{32}$P]dATP (2.5 μCi), deoxyguanosine triphosphate, deoxycytidine triphosphate, and deoxythymidine triphosphate. When indicated, 4 μg of p6 was added. After incubation at 30°C for 10 min, the reaction was stopped by addition of EDTA to 10 m*M* and SDS to 0.1% and by heating at 68°C for 10 min. After treatment with proteinase K, unincorporated [α-$^{32}$P]dATP was removed from the samples as indicated for the initiation reaction and subjected to electrophoresis in 1% agarose gels (*28*); gels were dried and autoradiographed. The arrow indicates the replicated DNA fragment.

tein p6–DNA complex was formed, phase changes of 4 or 12 bp completely abolished the ability of p6 to promote the initiation reaction and the transition to elongation. P6 activity was restored by in-phase insertions of 24 bp. These results suggest that, to start replication, the initiation proteins interact with p6 or recognize a precisely located conformational change in the DNA induced by p6. In addition, the fact that the initiation reaction and the transition to elongation are equally affected by the different phase changes may reflect the coupling of both steps, that is, the protein p6–DNA complex may not stimulate the transition step unless it has also stimulated the initiation reaction.

Formation of DNA–multimeric protein complexes at the replication origins of linear double-stranded DNA genomes with terminal proteins has been found in adenovirus (*6*), *B. subtilis* phage ϕ29 (*7*) and *Escherichia coli* phage PRD1 (*23*). The complex formed by the viral p6 at the ϕ29 replication origins is proposed to be a right-handed superhelix of DNA that tightly wraps around a multimeric protein p6 core interacting through the DNA minor groove. The novel features of this complex pose interesting questions concerning the structure and behavior of DNA. The p6 recognition of ϕ29 replication origins seems to rely only on the structural ability of the DNA to follow the path imposed by the p6 core (*11*). Furthermore, in contrast to other nucleoprotein complexes involved in the initiation of replication, p6 restrains positive supercoils in the DNA. Since the replication origins of ϕ29 were topologically unconstrained, at least in the in vitro system used, the restraining of positive supercoils, instead of having a global effect on the DNA molecule, must have a local effect that would be functional only when it is correctly positioned. P6 may be involved in the formation of a topological microdomain at the replication origin that would facilitate the local unwinding of DNA.

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21. Site-directed mutagenesis was done on the single-stranded form of the M13mp8 derivative mID13 [J. Gutiérrez, thesis, Universidad Autónoma de Madrid (1987)]. This form contains the terminal 125 bp of the right ϕ29 DNA end in the same construction as that in the M13 derivative G7 (*20*), except that the insert is inverted. The 39th position of the right terminal fragment, T, was changed to a G, to produce a Nco I restriction site, by hybridization of the appropriate oligonucleotide with a mismatch at the indicated position [M. J. Zoller and M. Smith, *Methods Enzymol.* **100**, 468 (1983)]. After primer extension with the Klenow fragment of DNA polymerase I and treatment with T4 DNA ligase, the mixture was used to transform competent *Escherichia coli* JM103 cells. The replicative form DNA isolated from plaques was analyzed by restriction analysis. Insertion of 4 bp was done by cleavage of the Nco I mutant replicative form DNA with Nco I filling-in the cohesive ends and religation. To insert 12 bp and 24 bp, we synthesized partially complementary oligonucleotides, CATGGATCCACT and CATGAGTGGATC for the 12-bp insertion and CATGGACCGACTATCCTCGAGAAG and CATGCTTCTCGAGGATAGTCGGTC for the 24-bp one, so that the 5′ protruding ends were compatible with the Nco I cohesive ends and, once inserted, gave rise to 12- and 24-bp insertions with a sequence almost identical to the wild-type sequence downstream from position 39. In the 12-bp insertion, C and G corresponding to positions 42 and 44 were changed to T and C, respectively, to generate a Bam HI restriction site. In the 24-bp insertion, T and C corresponding to positions 51 and 56 were changed to C and G, respectively, to generate a Xho I restriction site. The appropriate synthetic oligonucleotides were mixed in equimolar amounts, heated to 60°C for 15 min and allowed to hybridize at room temperature. Insertion into the Nco I site of the Nco I mutant replicative form DNA was done with standard protocols. After transformation, replicative form DNA was isolated from plaques and analyzed by restriction analysis. In all cases, sequences were confirmed by DNA sequencing (*24*) [F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977)]. The corresponding Nar I to Dra I DNA fragments of 362 (+insertion) base pairs were used for the DNase I footprinting experiments and the Dra I B fragments of 1741 (+insertion) base pairs for the in vitro replication assays.
22. The ϕ29 DNA terminal fragments lacking the terminal protein are active templates in the initiation

reaction, although their activity is lower than that of fragments containing the terminal protein [J. Gutiérrez, J. A. García, L. Blanco, M. Salas, *Gene* **43**, 1 (1986)].
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29. We thank J. M. Lázaro for supplying the ϕ29 purified proteins used in this work. This investigation has been aided by research grant 5 R01 GM 27242-10 from the NIH, by grant PB87 0323 from the Dirección General de Investigación Científica y Técnica, and by a grant from Fundación Ramón Areces. M. Serrano was recipient of a fellowship from Fondo de Investigaciones Sanitarias.

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# Shared Human T Cell Receptor $V_\beta$ Usage to Immunodominant Regions of Myelin Basic Protein

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**Multiple sclerosis (MS) may be an autoimmune disease mediated by T cells specific for a myelin protein. Investigations have demonstrated myelin basic protein (MBP)–reactive T cells that were activated in vivo in MS patients, suggesting that MBP may be a target antigen in MS. The variable (V) region of the T cell receptor (TCR) β chain was examined among 83 T cell lines from both MS patients and healthy subjects that were reactive with the immunodominant region of human MBP (residues 84 to 102) or with a second immunodominant region of MBP (143 to 168). $V_\beta$17 and to a lesser extent $V_\beta$12 were frequently used in recognition of MBP(84–102) among different individuals. In contrast, $V_\beta$17 was very infrequent among lines reactive with MBP(143–168). These data demonstrate shared TCR $V_\beta$ gene usage for the recognition of immunodominant regions of the human autoantigen MBP. Such TCR structures may be used as targets for specific immunotherapy in MS.**

MULTIPLE SCLEROSIS (MS) IS A chronic inflammatory disease of the central nervous system characterized by prominent T cell and macrophage infiltrates and demyelination. The pathogenesis of MS is thought to arise from autoreactive T cells specific for a myelin protein that initiate the inflammatory process (*1, 2*). The recent demonstration of in vivo–activated myelin basic protein (MBP)–specific T cells in MS patients implicates MBP-reactive T cells in the pathogenesis of the disease in some individuals (*3*). Two regions of human MBP located between residues 84 to 102 and 143 to 168 were recently identified to be immunodominant (*4, 5*). MS patients have a higher frequency of T cells reactive with MBP(84–102) in their blood than do controls. Reactivity to MBP(84–102) is associated with DR2, whereas reactivity to MBP(143–168) is associated with DRw11 (*4*).

Experimental autoimmune encephalomyelitis (EAE) is the primary animal model for MS and can be induced by immunization with MBP in adjuvant or by passive transfer of $CD4^+$ T cells reactive with MBP (*2, 6*). T cells that induce EAE in both mice and rats are specific for immunodominant MBP peptides presented by particular major histocompatibility complex (MHC) class II molecules and recognize MBP with specific T cell receptor (TCR) variable (V) regions of the β and α chains (*7–9*). In B10.PL mice encephalitogenic T cells recognize an $NH_2$-terminal MBP peptide (residues 1 to 9) presented by an $H\text{-}2^u$–encoded MHC molecule (*7*). The majority of T cells recognizing this MHC-peptide complex use $V_\beta$8.2 and $V_\alpha$2 or $V_\alpha$4 (*8*). In Lewis rats TCR segments homologous with the mouse $V_\beta$8.2 and $V_\alpha$2 genes have been found in encephalitogenic T cells specific for MBP(68–88) and I-A (*9*). Administration of a $V_\beta$8.2-specific monoclonal antibody (MAb) is effective in treating murine EAE, and immunization with $V_\beta$8.2-specific peptides ameliorates EAE in the Lewis rat (*8, 10*). Thus, we analyzed in humans which $V_\beta$ regions were used among T cells recognizing immunodominant regions of MBP.

TCR $V_\beta$ usage of MBP-reactive T cell lines was determined by the polymerase chain reaction (PCR) amplification of cDNA with TCR $V_\beta$1 to $V_\beta$20 primers, followed by Southern (DNA) blotting, and hybridization with an internal TCR $C_\beta$ probe (*11–14*). Particular care was taken to prevent contamination of samples. Amplified and nonamplified samples were handled separately; reagents were divided into aliquots and tested for the presence of amplified material, and negative controls were included for all experimental steps (RNA isolation, cDNA synthesis, and PCR amplification).

Two series of experiments were performed to test the validity of this approach. First, we demonstrated that all primers except $V_\beta$20 were able to amplify cDNA from peripheral blood T cells (Fig. 1). Second, the specificity of PCR amplification was examined by analysis of $V_\beta$ gene usage in 69 independent T cell clones previously established by single-cell cloning with mitogen (phytohemagglutinin, PHA) and interleukin-2 from a normal subject and an MS patient (*15*). Because of the high cloning efficiencies, these clones provide a representative analysis of $V_\beta$ gene usage among peripheral blood T cells. TCR $V_\beta$ gene usage could be determined for 65 of 69 (94.2%) of these T cell clones, indicating that a large proportion of the TCR $V_\beta$ repertoire was recognized by our $V_\beta$ primers. Whereas 58 of these clones (84%) were positive for a single $V_\beta$, seven clones (10.1%) were double-positive, possibly because of the presence of two rearranged and expressed TCR genes or the presence of two cell populations in the sample.

TCR $V_\beta$ gene usage was analyzed in a total of 64 MBP(84–102)–reactive T cell lines and 19 MBP(143–168)–reactive lines established from five patients with definite early relapsing-remitting MS (*4*) and five control subjects. Thirty-one T cell lines from patient Hy reacted with MBP(84–102) (patient 1, Fig. 2). The $V_\beta$17 segment was used in 24 (77.4%) T cell lines, whereas other cell lines used $V_\beta$4, $V_\beta$7, or $V_\beta$14. Thus, $V_\beta$17 is the major TCR $V_\beta$ structure used to recognize MBP(84–102) in this MS patient. $V_\beta$17 usage was also found in T cell lines from the four other patients examined (Fig. 2) and was present in 6 of 20 T cell lines. $V_\beta$12 was also used frequently and was found in 7 of 20 T cell lines reactive with MBP(84–102) (Figs. 1 and 2). This $V_\beta$ is homologous to the mouse $V_\beta$8.2 (*16*), which is the predominant TCR $V_\beta$ used among encephalitogenic T cells in mice and rats (*8, 9*).

We examined TCR $V_\beta$ gene usage among T cell lines reactive with MBP(84–102) in five healthy individuals (Fig. 2). All five cell lines from subject Rt, who was $DR2^+$, were

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