Our results suggest that TFIIID may have a broader function in gene expression than previously appreciated (21, 22). The possibility that a transcription factor is shared by pol B and pol C correlates well with previous indications that upstream activating factors stimulate transcription by the B and C enzymes (2, 23). These upstream activator proteins may act via TFIIID (22, 24). Participation of the TATA factor in the transcription of pol C genes may not be restricted to the U6 (and 7SK) genes, as upstream TATA-like elements have been found in a number of other genes transcribed by pol C (1).

In pol B genes, the interaction of TFIIID with the TATA element is thought to promote preinitiation complex formation by favoring subsequent binding of TFIIIB (or a preformed pol B–TFIIB complex) (22, 25). In the case of the U6 gene, TFIIID may favor the assembly of TFIIB that, by itself, does not bind DNA (16, 18). A functional and evolutionary relationship may therefore exist between TFIIIB and TFIIID or some other general pol B factor. The relatedness of class C and class B transcription factors would thus parallel the close evolutionary relationship of pol B and pol C, as these two enzymes were found more closely related to each other than to enzyme A (26).

REFERENCES AND NOTES
10. TFIIIB activity was purified successively by phosphocellulose, heparin-agarose, and Cl-agarose chromatography as described (8), then subjected to fast protein liquid chromatography (FPLC) on a Superose 12 column (Pharmacia) equilibrated in 20 mM Hepes-KOH, pH 7.5, 0.5 M EDTA, 10 mM 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 10% glycerol, and 0.05 M NaCl (250 μl) eluted with the same buffer was assayed for transcription factor activity with the rRNAΔb or the U6 genes. Molecular sizes of proteins were estimated with size markers (Bio-Rad protein standards). Transcription mixtures (8 μl) received 7-μl aliquots of different column fractions as indicated, and contained 120 mM KCl (final concentration), purified pol C (50 ng), and rTaqplasmid DNA (150 ng) (9). For rRNA synthesis, the mixtures were supplemented with factor Tau (75 ng). After a 50-min incubation at 25°C, transcripts were analyzed for polyacrylamide-urea gel electrophoresis and autoradiography (8).

11. This nucleic acid cleaves phenol-extracted U6 RNA (114 nt) in transcription buffer. The activity requires Mg2+ ions and is inhibited at high salt concentrations (F. Margottin and A. F. Burnol, unpublished data). A similar activity that selectively trims mouse U6 RNA has been described [D. I. Lee, H. Hirai, S. Natori, K. Sekimizu, J. Biochem. 110, 526 (1989)].

12. F. Margottin and G. Dujardin, unpublished observations.
15. For S1 mapping, the RNA from 200-μl transcription reactions was annealed to a 191-nt Taq I-Eco NI fragment labeled at the 5’ end on the coding strand (8), and samples were processed according to a standard S1 mapping procedure [T. Maniatis, E. F. Fritsch, J. Sambrook, Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1983)]. Partially purified U6 RNA consisted of a preparation of yeast RNA enriched for small RNA species by gel electrophoresis and contained predominantly 5S RNA.
20. In preliminary experiments with a U6 gene that harbors the B-block, we found that peak 2 fractions (TFIID) were still required for U6 RNA synthesis, even in the presence of Tau (F. Margottin and A. F. Burnol, unpublished observations).
27. We thank A. F. Burnol for her help at the final stage of this work. C. Carle, O. Lefebvre, M. Riva, and especially A. Ruet for constant discussions and various materials, and L. Faux for y1D expression vector. G.D. was on secondment from the Centre National de la Recherche Scientifique. Supported by a grant from the Ministère de la Recherche et de la Technologie (F.M.).

28 August 1990; accepted 9 November 1990

A Genetic Model for Interaction of the Homeodomain Recognition Helix with DNA

STEVEN D. HANES AND ROGER BRENT

The Bicoid homeodomain protein controls anterior development in the Drosophila embryo by binding to DNA and regulating gene expression. With the use of genetic assays in yeast, the interaction between the Bicoid homeodomain and a series of mutated DNA sites was studied. These experiments defined important features of homeodomain binding sites, identified specific amino acid–base pair contacts, and suggested a model for interaction of the recognition α-helices of Bicoid and Antennapedia-class homeodomain proteins with DNA. The model is in general agreement with results of crystallographic and magnetic resonance studies, but differs in important details. It is likely that genetic studies of protein-DNA interaction will continue to complement conventional structural approaches.

THE GENE bicoid ENCODES A PROTEIN morphogen (Bicoid) that is required for anterior development (1). Like many regulatory proteins important for development, Bicoid contains a 60–amino acid sequence known as the homeodomain (2, 3). Bicoid exerts its effects, in part, by activating expression of zygotic genes such as hunchback and orthodenticle (4). Bicoid binds to the sequence TCTAATCCCT and close variants repeated in the 5’ regulatory region of hunchback (5). The Antennapedia (Antp) class of homeodomain proteins, such as those encoded by Antennapedia, fushi tarazu, and Ultrabithorax, bind the sequence TCAATTAAT, which was first identified upstream of engrailed (6–8), a gene involved in segmentation.

The homeodomain contains a structure similar to the helix-turn-helix motif of prokaryotic transcriptional repressors (9, 10). Recognition of specific DNA sites by homeodomain proteins depends on the second α-helix (recognition helix) of this motif (11, 12). Bicoid and Antp-class proteins use Lys or Gln, respectively, at position 9 of the recognition helix (Fig. 1A) to distinguish between related binding sites (11, 12). When Lys9 in the Bicoid recognition helix is replaced by Gln, the mutant protein (Bicoid-Q9) no longer recognizes Bicoid sites, but instead recognizes Antp-class sites (11). Bicoid and Antp-class proteins expressed
in yeast stimulate transcription of target genes that contain functional binding sites (11, 13). In this report, we measured Bicoid-dependent transcription from GALI-lacZ constructs that contained multiple copies of wild-type or mutant binding sites. We changed nucleotides in the Antp-class binding site to those found at equivalent positions in the Bicoid binding site (and vice versa) to generate a series of sites with switched specificities for protein binding. This strategy allowed us to delineate the minimal site requirements for homeodomain recognition and to identify base pairs in each site that determine which class of protein is bound.

Experiments (Table 1) designed on the basis of an alignment of binding sites as they occur upstream of regulated genes (Fig. 1, B and C) indicated the importance of base pairs 7 and 8, but did not produce sites with switched specificities. This prompted us to devise an alternative alignment with the use of the complementary strand of the Antp site (Fig. 1D).

On the basis of the new alignment, we replaced CCC at positions 7, 8, and 9 in the Bicoid site by TGA, which is found at these positions in the Antp site (mutant Bicoid site 9, Table 2A). The mutant site had switched specificity; it was recognized by Bicoid-Q9, but not by wild-type Bicoid. We then tested a series of sites to determine the individual contributions of base pairs 7, 8, and 9 (sites 10 to 17). Our results showed that base pair 7 was critical for site recognition by Bicoid-Q9 (see especially sites 9 and 15 to 17). In addition, base pair 8 (sites 12 to 14 and 24) (14) and base pair 9 (sites 9 to 12) influenced recognition by Bicoid-Q9; purines were preferred over pyrimidines on the upper strand.

We performed reciprocal experiments to switch the specificity of an Antp site to that of a Bicoid site (Table 2B). An Antp site that contained CC in positions 7 and 8 was recognized by wild-type Bicoid but not Bicoid-Q9 (site 18). Results with sites 18 to 25 showed that base pair 7 was also critical for recognition by wild-type Bicoid (see especially sites 18 versus 22) and that pyrimidines (preferably cytosine) were favored over purines on the upper strand at base pair 8 (sites 5 and 18 to 21).

We draw a number of conclusions from the above experiments. In all sites with switched specificity, the identity of base pair 7 is decisive; wild-type Bicoid requires C,G, while Bicoid-Q9 requires T,A, indicating that Bicoid and Antp-class homeodomain

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**Table 1.** Recognition of mutant binding sites by wild-type Bicoid and altered specificity Bicoid-Q9 proteins. Yeast cells were cotransformed with plasmids that encoded Bicoid activator proteins and plasmids that contained a target gene that carried the binding sites to be tested (27). Bicoid proteins were expressed as fusions to the *Escherichia coli* LexA protein (11, 27). DNA binding-dependent stimulation of GAL1-lacZ target genes was measured as described (11). Designations are: (+++) >100 units of β-galactosidase activity; (+ +) 20 to 100 units; (+) 2 to 20 units; (+/-) 0.1 to 2 units; and (-) <0.1 unit. The number of binding sites for constructs 1 to 7 was between 6 and 14.

| Bicoid | 123456789 | | Bicoid-Q9 | |
|-------|------------|----------|
| Wild-type Bicoid site | TCTAACTCC | +++ (106) |
| Mutant Bicoid site (1) | TCTAACTTC | <0.1 |
| Mutant Bicoid site (2) | TCTAAAGC | <0.1 |
| Mutant Bicoid site (3) | TCTAAATCC |< (0.1) |
| Mutant Bicoid site (4) | TTCTAATTC |< (0.1) |
| Mutant Bicoid site (5) | TTCTAATCC |++ (75)* |
| Mutant Bicoid site (6) | TTCTAATCC |< (0.1) |
| Mutant Bicoid site (7) | TTCTAAACCC |< (0.1) |
| Wild-type Antp site | ATTTAATTGA |< (0.1) |
| Mutant Antp site (8) | ATTTAATTGA |++ (97) |

*The target plasmid that bore mutant site 5 contained a large number of binding sites, 14, which may explain the high levels of activation compared to sites 18 and 21 (Table 2).

**Table 2.** Switching the specificity of Bicoid and Antp binding sites. Recognition of mutant sites by Bicoid and altered specificity Bicoid-Q9 was determined as described in Table 1. Here, each target gene was constructed (28) so that it contained exactly six copies of each binding site oriented 5' to 3' as shown in the table. Designations for units of β-galactosidase activity (+, -) are as in Table 1.

<table>
<thead>
<tr>
<th>Bicoid</th>
<th>123456789</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type Bicoid site</td>
<td>TCTAACTCC</td>
</tr>
<tr>
<td>Mutant Bicoid site (9)</td>
<td>TCTAAATTGA</td>
</tr>
<tr>
<td>Mutant Bicoid site (10)</td>
<td>TCTAAATTG</td>
</tr>
<tr>
<td>Mutant Bicoid site (11)</td>
<td>TCTAAATTG</td>
</tr>
<tr>
<td>Mutant Bicoid site (12)</td>
<td>TCTAAATTG</td>
</tr>
<tr>
<td>Mutant Bicoid site (13)</td>
<td>TCTAAATTG</td>
</tr>
<tr>
<td>Mutant Bicoid site (14)</td>
<td>TCTAAATTG</td>
</tr>
<tr>
<td>Mutant Bicoid site (15)</td>
<td>TCTAAATTG</td>
</tr>
<tr>
<td>Mutant Bicoid site (16)</td>
<td>TCTAAATTG</td>
</tr>
<tr>
<td>Mutant Bicoid site (17)</td>
<td>TCTAAATTG</td>
</tr>
</tbody>
</table>

**Table 2.** Switching the specificity of Bicoid and Antp binding sites. Recognition of mutant sites by Bicoid and altered specificity Bicoid-Q9 was determined as described in Table 1. Here, each target gene was constructed (28) so that it contained exactly six copies of each binding site oriented 5' to 3' as shown in the table. Designations for units of β-galactosidase activity (+, -) are as in Table 1.

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proteins discriminate between their sites by an interaction between recognition helix residue 9 and base pair 7.

Base pairs 8 and 9 also influence recognition, perhaps by providing weak contacts that cannot be strictly base-specific, as several combinations of base pairs are permissible. For example, at position 8, Bicoid-Q9 tolerates A, G, and, to a lesser extent, T (sites 9, 13, and 14), while wild-type Bicoid tolerates C and, to a lesser extent, T (sites 18 and 21).

Base-specific contacts to positions 1 and 2 are not critical for binding, because Bicoid and Bicoid-Q9 tolerate either T or C at position 2 (for example, site 18 versus a wild-type Bicoid site). In addition, a site in which positions 1 and 2 are CG instead of TC is still bound by Bicoid (Fig. 1B, site A1) (5).

The TAAT motif, which is conserved in nearly all homeodomain binding sites (3), is not used to discriminate between Bicoid and Antp binding sites, because complete switches in specificity are obtained with sites in which the TAAT is unchanged (for example, sites 9 and 18). Its importance, however, is inferred from alkali interference experiments (15), in vitro binding studies (7), and from our own experiments, showing that T at position 6 is crucial for recognition by both Bicoid and Bicoid-Q9 (sites 7 and 8). It is likely that the TAAT is necessary to distinguish these sites from nonspecific DNA. Thus, the homeodomain binding site consists of two subsites, a common TAAT core element and specificity-determining bases that lie 3' to it. In the sites examined here, the closest base pair to the 3' end of the TAAT element (TAATG) contributed the most to binding specificity. The fact that switched specificity sites are obtained with nonsymmetrically disposed base substitutions in one-half of the site suggests that each site is bound by a single protein monomer. This is consistent with recent biochemical studies (15) and with the general lack of symmetry in homeodomain binding sites (7).

Our results strongly suggest that interactions between the recognition helix and DNA occur in the major groove. This conclusion follows from the fact that Bicoid-Q9 distinguishes between T:A and A:T at position 7 (sites 9 versus 16). These base pairs display distinct functional groups in the major groove, but almost identical groups in the minor groove (16). Similarly, wild-type Bicoid distinguishes between base pairs C:G and G:C at positions 7 and 8 (sites 17 versus 18), which are nearly indistinguishable in the minor groove (16). Finally, contacts made to base pair 6 in each site are also likely to be in the major groove, because Bicoid proteins distinguish T:A from A:T base pairs at this position (sites 7 and 8 versus wild type). On the basis of known contacts made by Lys92 in λ cro (17), Lys4 and Gln44 in λ repressor (18), and Glu28 in 434 repressor (19), we propose the interactions depicted in Fig. 2. Here, Lys9 contacts guanine of the C:G base pair and Gln9 contacts adenine of the T:A base pair.

The fact that amino acid 9 in both proteins interacts with the same base pair in both Bicoid and Antp sites implies that their recognition helices contact DNA in the same way. The Antp-class protein, fushi tarazu (Ftz), which has Gln at position 9, makes an equivalent contact to base pair 7 (20), suggesting that Bicoid and Antp-class proteins recognize DNA in a fundamentally similar way.

What other amino acids in the recognition helix contact DNA? We reasoned that contact amino acids should be near residue 9, on the same side of the α-helix and able to hydrogen-bond with base pairs in DNA. If such amino acids were conserved, they might contact conserved features of each site. For Bicoid, candidate residues that are conserved include Lys5, Asn10, and Arg12 (Lys5, Asn11, and Arg13 in Antp) (10) (Fig. 1A). A contact by Lys5 seemed unlikely, because replacement by Ala did not affect binding (11).

We tested whether Asn10 and Arg12 made essential DNA contacts by introducing Asn10→Ala, Asn10→Gln, or Arg12→Ala substitutions into wild-type Bicoid. As in previous experiments (11) (see also legend to Table 1), Bicoid proteins were expressed as fusions to the bacterial LexA protein. All three mutants were defective for recognition of both the Bicoid site and a switched-specificity Antp-class site (Table 2). The mutant proteins were apparently specifically defective in homeodomain-DNA binding; they were stably expressed, as demonstrated by immunoblot analysis, and they activated LexA operator-containing target genes, indicating that their nuclear transport, oligomerization, and gene activation functions were maintained (20). While such loss of function experiments cannot establish precise contacts, the results suggest that Asn10 and Arg12 contact conserved features of the two sites. If these contacts are sequence-specific, they would be made to the conserved TAAT element, which would indicate that the recognition helix is aligned with its NH2-terminus toward the 3' end of the binding site (Fig. 3). Our model suggests that like prokaryotic helix-turn-helix proteins, the homeodomain recognition helix makes specific DNA contacts in the major groove. However, the geometry of this interaction is quite different; unlike prokaryotic proteins, residues toward the COOH-terminus make these contacts. Our experiments show that Lys9 of the Bicoid recognition helix contacts base pair 7 of the Bicoid site (TCTAATCCC), while Gln substituted at this position (as in Antp-class proteins) contacts the equivalent base pair 7 of the Antp site. The Bicoid binding site is the same for both (16).
Table 3. Elimination of DNA binding by mutations in Asn10 and Arg12. Mutant proteins (29) were tested for recognition of Bicoid binding sites (TTCTAATCC) and switched-specificity (sw) Antp-class sites (site 18, ATTTAATCCA) as described in Table I. Results given in units of β-galactosidase activity have been normalized to activity obtained when assayed on LexA operator-containing target genes.

<table>
<thead>
<tr>
<th>Producer plasmid</th>
<th>Target plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bicoid site</strong></td>
<td><strong>Antp site (sw)</strong></td>
</tr>
<tr>
<td>Bicoid wild-type</td>
<td>119</td>
</tr>
<tr>
<td>Asn10 → Ala</td>
<td>6</td>
</tr>
<tr>
<td>Asn10 → Ala</td>
<td>2</td>
</tr>
<tr>
<td>Arg12 → Ala</td>
<td>3</td>
</tr>
</tbody>
</table>

Base pair in the Antp-class site (TTCTAATCCA). They show that conserved residues Asn10 and Arg12 contact DNA and suggest that the recognition helix is aligned COOH-terminus to NH2-terminus (C to N), 5’ to 3’ with the binding site. In this model, NH2-terminal residues 1 and 2 of the recognition helix do not contact DNA, and we suggest that their partial sequence conservation (3) may reflect interactions with a limited set of cellular proteins that modulate homeodomain DNA binding or gene regulation.

This picture is in good agreement with a recently reported crystal structure of an Engrailed-DNA complex (21) and a model of an Antennapedia-DNA complex proposed from nuclear magnetic resonance (NMR) experiments (22). In all three models (23), the COOH-terminus of the recognition helix inserts in the major groove, and the helix is tilted in a C to N, 5’ to 3’ configuration. In all three models, Asn10 and Arg12 (Asn51 and Arg53 in Engrailed and Antp) are in position to contact DNA. In the cocystal, Asn10 contacts a base in the core of the site (TAAT), while Arg12 makes a backbone contact outside of the TAAT. In the NMR model, contacts by Asn10 and Arg12 were not observed, perhaps because not all of the protein-DNA NOEs (nuclear Overhauser effects) were identified.

There are important differences between the models. In each model, the side chain of recognition helix residue 9 (Gln50 in Engrailed and Antp) makes a different major groove contact. In the cocystal, this Gln is in position to hydrogen bond with the adenine of base pair 7, as we have deduced, but instead makes van der Waals contact with thymine of base pair 8 (TAATNN).

We think it unlikely that any such contact determines specificity in vivo, because our functional assays show sites that do not have thymine at position 8 are recognized by Bicoid-Q9 (sites 9 to 12 and 14) and, conversely, sites that do have thymine at position 8 but have the wrong base at position 7 are not recognized by Bicoid-Q9 (sites 1 and 2). Moreover, we note that the proposed bidentate hydrogen bond contact between Gln and adenine (Fig. 2) would contribute more free energy to binding. As the authors have suggested (21), the contact observed in the cocystal might have been caused by distortions in the DNA caused by adventitious binding of a second protein monomer to the end of the fragment; alternatively, it may result from the solvent used to induce crystal formation, or it may be a nonspecific contact observed only at the high protein concentration in the cocystal.

In the NMR model, residue 9 contacts base pair 7, but the site contains G:C instead of T:A at this position. This interaction may be specific, but, because cytosine can only make a single hydrogen bond contact with Gln, it is unlikely to be as energetically favorable as an interaction with adenine. In fact, Fz, which contains Gln at residue 9, binds in vitro to sites with G:C at position 7 (TAATG) about one-tenth as strongly as it binds to sites with T:A at this position (TAATT) (24), and at the protein concentration found in vivo, neither Bicoid-Q9 (site 17) nor Fz (20) recognizes the G:C-containing site. On the basis of these results, we suggest that, in Drosophila, T:A will be the preferred base pair.

Finally, both the cocystal structure and NMR model show several DNA contacts not addressed in our experiments [for example, a contact between Ille65 in the recognition helix (Ille67 in Engrailed and Antp) and the TAAT, and contacts made by amino acids outside of the recognition helix]. These findings illustrate an important shortcoming of our approach; that is, it cannot detect interactions that we do not explicitly investigate. However, when used to test specific structural ideas, we believe that simple genetic and biochemical assays will continue to provide independent insight into structural issues (25) and occasional correction (26) of conclusions derived from conventional structural methods.

REFERENCES AND NOTES
2. J. W. Gehring, Science 236, 1245 (1987); S. Ha 
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the transcription start site are 5' to 3' as shown in Table 2. β-Galactosidase activity for mutant sites 9 to 25, wild-type Bicoid, and wild-type Antp sites are directly comparable because the number and orientation of binding sites were identical in each construct as confirmed by DNA sequencing. The general form for each pair of oligonucleotides is as follows:

**Bicoid sites:** upper strand, 5'-TGGAAT(TCTATT-NNT）T3'; lower strand, 5'-TCAAGA(TTATTAA)G3'.

**Antp sites:** upper strand, 5'-TGGAAT(TCTATT-NNT）T3'; lower strand, 5'-TCAAGA(TTATTAA)G3'.

29. Antp→Ala; Antp→Ala; and Antp→Gln substitutions in the Bicoid recognition helix were created with an overlapping polymerase chain reaction (PCR) strategy as described [R. M. Horton et al., BioTechniques 8, 538 (1990)]. The bicoid-containing Eco RI fragment of pSh11-1 (11) was subcloned into pUC119 [J. Vieira and J. Messing, Methods Enzymol. 153, 3 (1987)]. Two inner oligonucleotide primers were used to introduce the mutations in two separate PCR reactions extending in opposite directions from the site of the base changes. The purified products of the first sets of reactions were annealed and used for a second round of PCR with only outer primers to amplify an intact Sal I–Sal I fragment, which was then recloned into the bicoid backbone in pUC119. Vent polymerase was used for all PCR reactions (New England Biolabs). Inner primer oligonucleotides were as follows:

**Antp→Ala:** 5'-GCTGGAATGATGTTTGAAGCGCGCTGACCC-3' and 5'-CGCAGGACGGGACCTAAAC-CAGATCTTCACCC-3'.

**Antp→Ala:** 5'-GCTGGAATGATGTTTGAAGCGCGCTGACCC-3' and 5'-CGCAGGACGGGACCTAAAC-CAGATCTTCACCC-3'.

**Antp→Gln:** 5'-GCTGGAATGATGTTTGAAGCGCGCTGACCC-3' and 5'-CGCAGGACGGGACCTAAAC-CAGATCTTCACCC-3'.

30. We are grateful to E. Golemis and A. Hochschild for helpful discussions and to J. Swatok, A. Ellington, A. Ephrussi, and R. Finley for comments on the manuscript. Supported by the Anne Fuller Fund (S.D.H.), the Pew Scholars Program (R.B.), and Hoehst AG.

**T Cell Receptor Peptide Therapy Triggers Autoregulation of Experimental Encephalomyelitis**

**HALINA OFFNER,* GEORGE A. HASHIM, ARTHUR A. VANDENBARK**

Encephalitogenic T cells specific for myelin basic protein share common V<sub>V</sub>8 peptide sequences in their T cell receptor (TCR) that can induce autoregulatory T cells and antibodies that prevent clinical signs of experimental autoimmune encephalomyelitis (EAE). It is not known, however, if TCR peptides can treat established disease. To test its therapeutic value, TCR-V<sub>Vβ</sub>8-39-59 peptide was injected into rats with clinical signs of EAE. This treatment reduced disease severity and speeded recovery, apparently by boosting anti-V<sub>Vβ</sub>8 T cells and antibodies raised naturally in response to encephalitogenic V<sub>Vβ</sub>8 T cells. These results demonstrate that synthetic TCR peptides can be used therapeutically, and implicate the TCR-V<sub>Vβ</sub>8-39-59 sequence as a natural idiotypic molecule involved in EAE recovery. Similarly, human TCR peptides may be effective in enhancing natural regulation of autoreactive T cells that share common V<sub>V</sub> genes.

**T**hrough mechanisms that are not yet fully understood, rat and mouse T cells that arise in response to immunization with guinea pig or rat basic protein (Gp-BP; Rt-BP) preferentially utilize the V<sub>Vβ</sub>8.2 gene and to a lesser extent the V<sub>Vβ</sub>2 gene in their TCR (1–6). The presence of common V<sub>V</sub> region sequences on encephalitogenic T cells allowed us (7) and others (8) to pre-immunize Lewis rats with synthetic TCR peptides to induce anti-receptor immunity. Our use of the synthetic V<sub>Vβ</sub>8 peptide corresponding to residues 39 to 59 completely protected the animals from the subsequent induction of clinical EAE. Similarly, most signs of EAE could be suppressed if the TCR-V<sub>Vβ</sub>8-39-59 peptide was given during the induction phase but prior to onset of clinical disease (9). The protective mechanisms involved both major histocompatibility complex (MHC) class I–restricted T cells (7) and antibodies (9) specific for the TCR-V<sub>Vβ</sub>8-39-59 peptide that appeared to be directed at a "processed" MHC-associated fragment of the natural TCR-V<sub>Vβ</sub> chain expressed on the surface of V<sub>Vβ</sub>8 T cells. Presumably, interaction of the regulatory T cell or antibody with the V<sub>Vβ</sub>8 encephalitogenic T cell perturbed membrane signaling pathways, thus altering effector cell functions.

Although many approaches have been described to prevent encephalomyelitis (EAE), effective treatment of established clinical signs has been much more difficult. To test its therapeutic potential, the TCR-V<sub>Vβ</sub>8-39-59 peptide was injected by several different routes into Lewis rats with moderate signs of EAE. As controls, rats with V<sub>Vβ</sub>8 T cell receptor were injected in parallel with a synthetic peptide corresponding to the TCR-V<sub>Vβ</sub>14-39-59 sequence (not utilized by encephalitogenic T cells), or saline.

Intradermal (id) injection of 50 μg of the TCR-V<sub>Vβ</sub>8-39-59 peptide in saline reduced the clinical severity of EAE from grade 3.5 (paralysis of hind limbs) in the control rats to grade 1.5 (wobbly gait) within 48 hours and to grade 0.2 (limp tail in some rats) within 72 hours (Fig. 1A). The TCR-V<sub>Vβ</sub>8-39-59 peptide treatment speeded overall recovery time from 6.3–6.6 days in control rats to 3.1 days (Fig. 1A). A lower dose (10 μg) of the id-injected peptide was only slightly less effective, with a recovery time of 4 days (Fig. 1A). The second route tested, subcutaneous injection of 100 to 500 μg of the TCR-V<sub>Vβ</sub>8-39-59 peptide in saline, produced a nearly identical resolution of clinical EAE, with a recovery time of 3.5 and 4 days respectively (Fig. 1B). A third regime, injection of the TCR-V<sub>Vβ</sub>8-39-59 peptide in complete Freund's adjuvant (CFA), also arrested disease progression within 24 hours and caused a rapid resolution of the remaining clinical signs from 6.5–6.6 days (controls) to 3.5 days (Fig. 1C).

The rapid clinical resolution of EAE after injection of the TCR-V<sub>Vβ</sub>8-39-59 peptide suggested a recall response similar to that induced in man by tetanus or rabies booster shots. Such a recall response would imply the presence of a preexisting immunity to the TCR-V<sub>Vβ</sub>8-39-59 peptide. Indeed, one might rationalize that the induction of V<sub>Vβ</sub>8 encephalitogenic T cells during EAE could stimulate regulatory T cells and antibodies directed at the V<sub>Vβ</sub>8 molecule and more specifically at the TCR-V<sub>Vβ</sub>8-39-59 peptide.

One simple method of assessing preexisting T cell responses in vivo is to measure ear swelling (delayed hypersensitivity [DH]) 24 to 48 hours after an id injection of antigen.