27. D. P. Gold *et al.*, *ibid.* **321**, 431 (1986).
28. G. W. Krissansen, M. J. Owen, W. Verbi, M. J. Crumpton, *EMBO J.* **5**, 1799 (1986).
29. Y. Yoshikai *et al.*, *Nature (London)* **312**, 521 (1984).
30. H. Band *et al.*, *Science* **238**, 682 (1987).
31. J. Borst *et al.*, *Nature (London)* **325**, 683 (1987).
32. P. Moingeon *et al.*, *ibid.*, p. 723.
33. L. L. Lanier *et al.*, *J. Exp. Med.* **165**, 1076 (1987).
34. M. S. Krangel, P. Devlin, S. Hata, H. Band, M. B. Brenner, unpublished results.
35. J. McLean and M. B. Brenner, unpublished results.
36. B. Toyonaga and T. W. Mak, *Annu. Rev. Immunol.* **5**, 585 (1987).
37. T. Quertermous, W. M. Strauss, J. J. M. van Dongen, J. G. Seidman, *J. Immunol.* **138**, 2687 (1987).
38. We thank H. Band and J. Leiden for samples of RNA and DNA, J. McLean and P. Devlin for technical assistance, J. Boss and J. Santos for useful suggestions, and J. Strominger for his continued interest in this work. Supported by grants NSF-DCB-8617540, ACS-NY-IM-425 and NIH-1-K01-AM01598. S.H. is on leave from the National Institute of Agrobiological Resources, Tsukuba Science City, Yatabe, Ibaraki 305, Japan.

14 September 1987; accepted 1 October 1987

Immunochemical Proof That a Novel Rearranging Gene Encodes the T Cell Receptor δ Subunit

HAMID BAND, FRANS HOCHSTENBACH, JOANNE MCLEAN, SHINGO HATA, MICHAEL S. KRANGEL, MICHAEL B. BRENNER

The T cell receptor (TCR) δ protein is expressed as part of a heterodimer with TCR γ, in association with the CD3 polypeptides on a subset of functional peripheral blood T lymphocytes, thymocytes, and certain leukemic T cell lines. A monoclonal antibody directed against TCR δ was produced that binds specifically to the surface of several TCR γδ cell lines and immunoprecipitates the TCR γδ as a heterodimer from Triton X-100 detergent lysates and also immunoprecipitates the TCR δ subunit alone after chain separation. A candidate human TCR δ complementary DNA clone (IDP2 O-240/38), reported in a companion paper, was isolated by the subtractive library approach from a TCR γδ cell line. This complementary DNA clone was used to direct the synthesis of a polypeptide that is specifically recognized by the monoclonal antibody to TCR δ. This complementary DNA clone thus corresponds to the gene that encodes the TCR δ subunit.

T CELL RECEPTOR (TCR) α and β genes rearrange in T cells and encode the variable part of the TCR αβ–CD3 complex. Unexpectedly, another gene, TCR γ (*1*), that rearranges specifically in T cells was also found. The search for the protein product of this gene led to the identification of a second T cell receptor composed of the TCR γ polypeptide, the CD3 proteins, and a previously unidentified polypeptide called TCR δ (*2*). This receptor is expressed on the cell surface of a subpopulation of functional peripheral blood lymphocytes (*3, 4*), thymocytes (*5–7*), and other cell types (*8–10*). To understand better the structure, diversity, and function of TCR γδ, we sought to characterize the TCR δ subunit. The TCR δ protein is serologically distinct from the TCR γ protein since it is not recognized by antisera raised against TCR γ–encoded sequences (*2, 6*). Moreover, the tryptic peptide map of TCR δ is distinct from that of TCR γ protein isolated from the same cell line (*11*).

We generated a monoclonal antibody (mAb) that is specifically reactive with the TCR δ subunit and we determined the size and extent of glycosylation of the TCR δ polypeptide. We present immunochemical evidence that a novel complementary DNA (cDNA) clone encodes the δ subunit of the T cell receptor.

The TCR γδ–CD3 complex from the PEER cell line (*4, 12*) was used as immunogen in the production of antibody-secreting hybridoma cell lines (Fig. 1). Hybridomas were screened both by surface binding in cytofluorographic analysis and by immunoprecipitation in SDS–polyacrylamide gel electrophoresis (SDS-PAGE) analysis of PEER cells. Two hybridoma supernatants (5A6 and 4A1) bound to the surface of PEER cells. After subcloning, one mAb (5A6.E9) was characterized further. This mAb bound to the surface of TCR γδ lymphocytes (PEER and IDP2) but failed to react with TCR αβ cells (HPB-MLT or JURKAT) or with non-T leukocytes (Fig. 1). Although the immunogen was composed of a complex of TCR γδ and CD3, the differential reactivity on TCR γδ cell lines suggested the mAb was not directed against CD3 determinants.

The specificity of the mAb was determined in immunoprecipitation studies under conditions that affect the association of the chains constituting the complex. For example, when ^{125}I-labeled IDP2 cells are solubilized in CHAPS detergent, TCR γ and δ and CD3 γ, δ, and ε subunits remain part of an associated complex immunoprecipitated by an antibody directed against CD3 (Fig. 2, lanes 3, and 4). However, if radiolabeled IDP2 cells are solubilized in 2% TX-100 detergent, TCR γδ and CD3 are largely dissociated, and immunoprecipitation with a mAb to CD3 results in selective isolation of the CD3 chains (Fig. 2, lane 5). Correspondingly, under these conditions mAb 5A6.E9 immunoprecipitates TCR γδ as a heterodimer, without associated CD3 chains (Fig. 2, lane 6). This observation provides the first direct evidence that TCR γ and TCR δ exist as a heterodimer even in

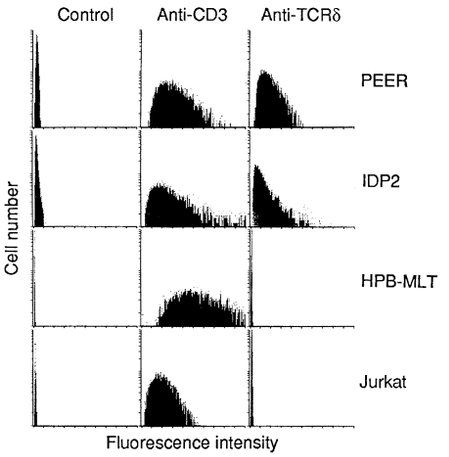

Fig. 1. Cytofluorographic analysis of T cell lines with anti-TCR δ1. TCR γδ cell lines PEER and IDP2 or TCR αβ cell lines HPB-MLT and JURKAT were stained with anti-TCR δ1 (Ig G1) culture supernatant, then with fluorescein isothiocyanate–conjugated goat antiserum to mouse Ig F(ab)'$_2$ fragments, and then analyzed on an Ortho cytofluorograph as described earlier (*2*). Control was the mAb secreted by P3X63.Ag8 hybridoma (P3), and the mAb to CD3 was anti-Leu 4 (*18*). The mAb to TCR δ was made as follows. PEER cells (1 g) were solubilized in 50 ml of 0.3% CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate) detergent and were immunoprecipitated with 1 μl of UCHT1 (*19*) ascites, 500 μl of mAb 187.1 culture supernatant, and 125 μl of a 10% suspension of fixed *Staphylococcus aureus* Cowan I strain for each immunization. Four intraperitoneal injections at 6-week intervals were carried out and were followed by a final boost of TCR γδ (without CD3) isolated by selective elution from the immune complexes with 2.5% TX-100 in 0.65*M* NaCl. After dilution to isotonic salt concentration, the eluted material was administered both intravenously and intraperitoneally, and 4 days after this final injection, the mice were killed and fusion was carried out as described earlier (*20*).

H. Band, F. Hochstenbach, J. McLean, S. Hata, M. S. Krangel, Division of Tumor Virology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.
M. B. Brenner, Division of Tumor Virology, Dana-Farber Cancer Institute, and Department of Rheumatology and Immunology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.

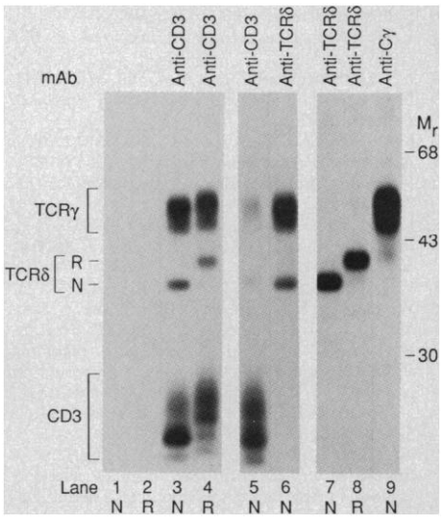

Fig. 2. Immunochemical analysis of the specificity of anti-TCR δ1. Surface ^{125}I-labeled IDP2 cells were immunoprecipitated with control mAb P3 (lanes 1 and 2), anti-Leu 4 (lanes 3 to 5), anti-TCR δl (lanes 6 to 8), or antiserum to cγ (lane 9) and were then resolved by SDS-PAGE and visualized by autoradiography. In CHAPS detergent, TCR γδ and CD3 remained associated and were immunoprecipitated as a complex by anti-Leu 4 (lanes 3 and 4). However, after solubilization in 2% TX-100 detergent, anti-TCR δ1 immunoprecipitated TCR γδ as a dimeric complex without CD3 (lane 6), and anti-Leu4 immunoprecipitated CD3 as a trimeric complex without TCR γδ (lane 5). After separation of the TCR γδ–CD3 component chains, anti-TCR δ1 immunoprecipitated TCR δ alone (lanes 7 and 8), while antiserum to Cγ immunprecipitated TCR γ alone (lane 9). For chain separation experiments (lanes 7 to 9), immunoprecipitates with anti-Leu 4 from CHAPS-solubilized IDP2 cells were boiled in 1% SDS and were then diluted with 10 volumes of 2% TX-100 and immunoprecipitated with anti-TCR δ1 or antiserum to Cγ. This follows procedures used earlier (*2*). N, nonreducing conditions; R, reducing conditions.

the non–disulfide-linked form. To determine whether mAb 5A6.E9 reacts with TCR γ, TCR δ, or a combinatorial determinant, we performed immunoprecipitation on separated chains. An immunoprecipitate obtained from radiolabeled CHAPS-solubilized IDP2 cells by using an antibody to CD3 was washed and boiled in 1% SDS to dissociate the individual species (TCR γ, TCR δ, and the CD3 subunits). After dilution with ten volumes of 2% TX-100, mAb 5A6.E9 specifically immunoprecipitated the 40-kD (TCR δ) species (Fig. 2, lane 7). When a portion of the same immunoprecipitate was analyzed under reducing conditions (Fig. 2, lane 8), a dramatic shift in SDS-PAGE mobility was observed, as previously reported to be characteristic of TCR δ on the IDP2 and PEER cell lines (*4*). In contrast, when the separated chains were immunoprecipitated with antisera to Cγ, the 55-kD species (TCR γ), but not the 40-kD species (TCR δ), was immunoprecipitated (Fig. 2, lane 9). On the basis of these biochemical and surface-binding studies, mAb 5A6.E9 will hereafter be referred to as anti-TCR δ1.

In addition to PEER and IDP2, anti-TCR δ1 also reacts with other TCR γδ cell lines, including MOLT-13 and PBL line 2. Whether anti-TCR δ1 reacts with a determinant encoded by a commonly used TCR δ V-gene segment or with a TCR δ C-gene segment is unknown. However, the observed binding clarifies the existence of the TCR δ polypeptide even in instances when cell-surface–labeled species are not clearly visualized. Similarly, the use of anti-TCR δ1 may help reevaluate the existence of TCR γγ homodimers suggested as present in some cases (*3, 8, 13, 14*). Moreover, the serological cross-reactivity suggests a relatedness among TCR δ proteins from different cell lines, a fact borne out by the results of peptide mapping studies (*15*).

Complementary DNA clones (for example, IDP2 O-240/38) isolated by the subtractive approach from the IDP2 cell line correspond to a candidate gene that may encode the TCR δ subunit (*16*). Genes cross-hybridizing with IDP2 group O cDNA clones are expressed and rearranged in TCR γδ lymphocytes but are typically not expressed and are often deleted in TCR αβ cells. Moreover, by sequence comparison with other TCR genes, these cDNA clones appear to be composed of novel V, D (?), J, and C gene segments. The IDP2 group O composite DNA sequence predicts a polypeptide with two potential asparagine-linked glycosylation sites and a peptide backbone of 31.3 kD (*16*). To determine the peptide backbone size and number of asparagine-linked carbohydrates that are present on the cell-surface IDP2 TCR δ polypeptide, we either treated gel-purified TCR δ with *N*-glycanase or mock-incubated it and reexamined it by SDS-PAGE (Fig. 3). Removal of *N*-linked carbohydrates resulted in a 5-kD decrease in size (from 40 to 35 kD). This result is suggestive of the presence of two (2.5 to 3 kD) *N*-linked glycans on the IDP2 TCR δ and correlates with the number of *N*-linked glycans predicted by the cDNA sequence (Fig. 3). The observed peptide size is in general agreement, but differs from that predicted by the cDNA clone by 3.7 kD.

In view of the reactivity of anti-TCR δ1 on IDP2 cells, the specificity for the TCR δ polypeptide, and the recognition of partially denatured (SDS-boiled) TCR δ, we tested the possibility that this mAb would recognize directly the polypeptide encoded by the candidate TCR δ cDNA clone. Thus, cDNA clone IDP2 O-240/38 was subcloned into the pGEM-3 expression vector downstream from the T7 promoter. Transcripts generated in vitro with T7 RNA polymerase were then used in a rabbit reticulocyte lysate system to direct the synthesis of protein in the presence of [^{35}S]methionine. After in vitro transcription and translation, the reaction products were boiled in 1% SDS, diluted with ten volumes of 2% TX-100, and then immunoprecipitated with either an isotype-matched control mAb or with anti-TCR δ1. Anti-TCR δ1 specifically immunoprecipitated a predominant species (34 kD) (Fig. 4, lane 4). No such band was observed in immunoprecipitates when control mAbs were used (Fig. 4, lane 3), when RNA transcripts were omitted (Fig. 4, lanes 1 and 2), or when TCR γ constructs were used. Thus, the radiolabeled species immunoprecipitated by anti-TCR δ1 corresponds to a polypeptide whose synthesis was specifically directed by the IDP2 O-240/38 cDNA clone. This 34-kD polypeptide is similar in size to the *N*-glycanase–treated IDP2 TCR δ chain (35 kD). The IDP2 0-240/38 clone lacks a natural ATG initiation codon as well as the leader sequence. There are two potential internal ATG codons (at residues 12 and 44) within the V region of this clone (Fig. 4A). Use of these codons to initiate synthesis could result in more than one polypeptide species, possibly accounting for the

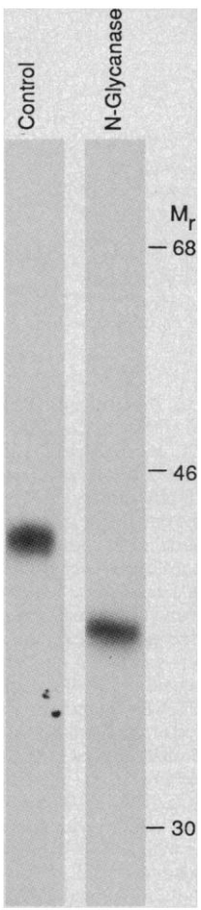

Fig. 3. *N*-linked glycosylation of the TCR δ polypeptide. Control lane is IDP2 TCR δ mock-incubated compared to *N*-glycanase–digested TCR δ. ^{125}I-labeled IDP2 cells were solubilized in 0.3% CHAPS and were immunoprecipitated with anti-Leu 4 and resolved by SDS-PAGE. TCR δ was eluted from a gel slice, boiled in 0.5% SDS with 0.1*M* 2-mercaptoethanol followed by *N*-glycanase (peptide N^4 [*N*-acetyl-β-glucosaminyl]asparagine amidase) (Genzyme) digestion (10 U/ml) in 0.17% SDS, 1.25% TX-100, 0.2*M* sodium phosphate buffer, *p*H 8.6, in a 30-μl reaction for 21 hours at 37°C (*21*). The digested or mock-incubated TCR δ were then resolved by SDS-PAGE (10% acrylamide) and visualized by autoradiography.

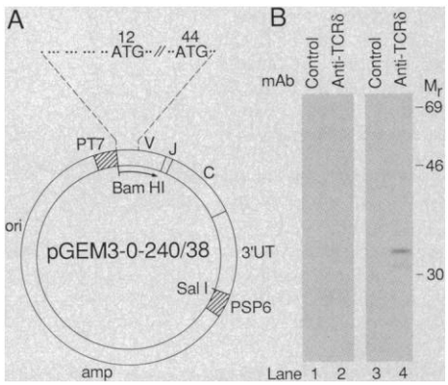

Fig. 4. Recognition of in vitro translated product of cDNA clone IDP2 O-240/38 by anti-TCR δ1. (**A**) pGEM3-O-240/38 construct used for in vitro transcription and translation. IDP2 O-240/38 cDNA clone (1.3 kb) begins within codon 7 of the composite group O sequence and includes the remaining coding region and most of the 3′ untranslated region (*16*). This insert was cleaved as a single Eco RI–Eco RI fragment from λgt10 arms by partial Eco RI digestion to prevent cleavage of the internal Eco RI site. This fragment was subcloned into the Bluescript+ vector (Stratagene). The clone was cleaved as a single Bam HI–Sal I fragment (ends are from the Bluescript vector polylinker) and was then directionally cloned into pGEM-3 (Promega Biotec) downstream from the T7 promoter to generate pGEM3-O-240/38. (**B**) Samples were immunoprecipitated with control mAb P3 (lanes 1 and 3) or with anti-TCR δ1 (lanes 2 and 4). Translations contained no RNA (lanes 1 and 2) or pGEM3-0-240/38–derived RNA template (lanes 3 and 4). pGEM3-0-240/38 plasmid was linearized with Sal I, and capped transcripts were synthesized with the use of T7 polymerase (*11*, *22*, *23*). Integrity and size of the transcripts were checked on a portion of the reaction mixture to which ^{32}P-labeled adenosine triphosphate had been added. A single RNA species of 1.3 kb was obtained. In vitro translation in the presence of [^{35}S]methionine was performed in a rabbit reticulocyte lysate (*24*). After in vitro translation, the samples were boiled in 1% SDS with 2 m*M* dithiothreitol followed by the addition of 10 volumes of 2% TX-100 in tris-buffered saline, *p*H 8.0. The samples were then immunoprecipitated with the above antibodies and analyzed by SDS-PAGE followed by fluorography (*25*).

minor species noted (Fig. 4, lane 4).

Taken together, the correlation between predicted and determined extent of glycosylation and peptide size, the selective expression and rearrangement in TCR γδ cells, and the direct serological recognition of the polypeptide encoded by IDP2 0-240/38, are compelling evidence that this candidate cDNA represents the gene encoding the IDP2 TCR δ subunit. The constant region of this cDNA clone is 79% identical at the nucleotide level to the recently described murine Cx gene (*17*), which may therefore correspond to the murine TCR δ equivalent. Cloning of both TCR γ and δ make it possible to more fully elucidate the expression, rearrangement, and diversity of the receptor. Moreover, mAbs against TCR δ that bind to the surface should facilitate functional studies and move us closer to understanding the role these cells play in the immune system.

REFERENCES AND NOTES

1. H. Saito *et al.*, *Nature (London)* **309**, 757 (1984).
2. M. B. Brenner *et al.*, *ibid.* **322**, 145 (1986).
3. J. Borst *et al.*, *ibid.* **325**, 683 (1987).
4. M. B. Brenner *et al.*, *ibid.* p. 689.
5. I. Bank *et al.*, *ibid.* **322**, 145 (1986).
6. A. M. Lew *et al.*, *Science* **234**, 1401 (1986).
7. D. M. Pardoll *et al.*, *Nature (London)* **326**, 79 (1987).
8. P. Moingeon *et al.*, *ibid.* **325**, 723 (1987).
9. D. R. Littman *et al.*, *ibid.* **326**, 85 (1987).
10. F. Koning *et al.*, *Science* **236**, 834 (1987).
11. M. S. Krangel *et al.*, *ibid.* **237**, 64 (1987).
12. A. Weiss, M. Newton, D. Crommie, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6998 (1986).
13. B. Alarcon *et al.*, *ibid.* **84**, 3861 (1987).
14. C. G. Ionnides *et al.*, *ibid.*, p. 4244.
15. F. Hochstenbach and M. B. Brenner, in preparation.
16. S. Hata *et al.*, *Science* **238**, 678 (1987).
17. Y.-H. Chien *et al.*, *Nature (London)* **327**, 677 (1987).
18. J. A. Ledbetter *et al.*, *J. Exp. Med.* **153**, 310 (1981).
19. P. C. Beverley and R. E. Callard. *Eur. J. Immunol.* **11**, 329 (1981).
20. M. B. Brenner *et al.*, *J. Immunol.* **138**, 1502 (1987).
21. A. L. Tarentino *et al.*, *Biochemistry* **24**, 4665 (1985).
22. P. A. Krieg and D. A. Melton, *Nucleic Acids Res.* **12**, 7057 (1984).
23. I. Hope and K. Struhl, *Cell* **43**, 177 (1985).
24. H. R. B. Pelham and R. J. Jackson, *Eur. J. Biochem.* **67**, 246 (1976).
25. W. J. Bonner and R. A. Laskey, *ibid.* **46**, 83 (1974).

26. We thank S. Speck for useful suggestions, J. Strominger for his continued support, and P. Beverley, N. Warner, and S. Ip for generously providing antibody reagents. This work was supported by grants ACS-NY-IM-425, NIH-1-KO1-AMO1598, NSF-DCB-8617540, 2-P60-AR36308, NIH-5RO1-AI15669, and NIH-SO7RR5526-24. H.B. is a research fellow of the Charles A. King Trust of Boston, MA. S.H. is on leave from the National Institute of Agrobiological Resources, Tsukuba Science City, Yatabe, Ibaraki 305, Japan.

14 September 1987; accepted 1 October 1987

The Adenovirus Major Late Transcription Factor Activates the Rat γ-Fibrinogen Promoter

LEWIS A. CHODOSH, RICHARD W. CARTHEW, JOHN G. MORGAN, GERALD R. CRABTREE, PHILLIP A. SHARP

The major late transcription factor (MLTF) is a 46-kilodalton polypeptide that specifically binds to and activates transcription from the major late promoter of adenovirus. The presence of this promoter-specific transcription factor in uninfected HeLa cell extracts suggests that MLTF is also involved in the transcription of cellular genes. This report demonstrates that MLTF specifically stimulates transcription of the rat γ-fibrinogen gene through a high-affinity binding site. Stimulation of transcription by MLTF was not dependent on the exact position of the MLTF binding site with respect either to the transcription initiation site or to adjacent promoter elements. These results suggest that one of the cellular functions of MLTF is to control γ-fibrinogen gene expression.

A WEALTH OF BIOCHEMICAL AND GENETIC evidence in eukaryotic cells suggests that the regulation of transcription initiation by RNA polymerase II is mediated by the interaction of multiple sequence-specific DNA-binding proteins with the promoter elements of these genes. One of these sequence-specific DNA-binding proteins, the major late transcription factor (MLTF or USF), was originally identified by its ability to selectively bind to and stimulate transcription from the adenovirus major late promoter (MLP) (*1*–*4*). Since this promoter-specific factor was found in extracts of uninfected HeLa cells, it was presumed that MLTF would also selectively stimulate transcription of cellular genes containing an appropriately positioned MLTF-binding site. We report here that MLTF selectively stimulates transcription from the rat γ-fibrinogen (γ-FBG) promoter.

Fibrinogen, an essential blood coagulation factor, is a 340-kD protein composed of two sets of three polypeptides (α, β, and γ), which are produced in the liver. These polypeptides are encoded by three separate genes whose transcription is coordinately regulated (*5*). Fibrinogen genes are also coordinately regulated along with a number of other genes in the mammalian liver during the acute phase response to infection, inflammation, and tissue injury. Recently, the promoter elements of the fibrinogen genes have been genetically identified in

L. A. Chodosh, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, and Harvard Medical School, Boston, MA 02115.
R. W. Carthew and P. A. Sharp, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.
J. G. Morgan and G. R. Crabtree, Stanford University School of Medicine, Stanford, CA 94305.