turnover in 9 min, will be regenerated. A standard minimum dosing interval of 10 to 15 min will allow for turnover, but because of the modest activity of this first artificial cocaine esterase, near stoichiometric amounts of antibody will be required.

To estimate the characteristics of an antibody useful at a clinically practical dose of <1 g, we assumed a dose of smoked crack cocaine of 100 mg, a peak pulmonary-venous cocaine concentration of 10 to 30 μM (*29*), and a 20-s duration of reaction (the transit time from pulmonary to CNS capillaries). This simple model neglects the volume of distribution for cocaine and the threshold concentration for a biological effect, which if included would reduce the kinetic requirements. Under these constraints, a catalytic antibody against cocaine should ideally have a turnover number >2 s^{-1} and a K_m < 30 μM to deactivate cocaine before significant partitioning into the CNS can occur. However, the protection afforded by the artificial esterase may not need to be complete to be useful, and a significantly less potent enzyme could nonetheless diminish the reinforcing effect of cocaine by reducing the rate of rise and peak concentration of cocaine. By promoting cessation of use and maintenance of abstinence, passive immunization with an anticocaine catalytic antibody could provide a window for appropriate psychosocial and relapse-prevention interventions.

REFERENCES AND NOTES

1. F. H. Gawin and E. H. Ellinwood, Jr., *N. Engl. J. Med.* **318**, 1173 (1988).
2. M. W. Fischman, *J. Clin. Psychiatry* **49** (suppl. 2), 7 (1988); J. Bergman, B. K. Madras, S. E. Johnson, R. D. Spealman, *J. Pharmacol. Exp. Ther.* **251**, 150 (1989); C. E. Johanson, in *NIDA Res. Monogr. 50* (1984), pp. 54–71.
3. N. E. Goeders and J. E. Smith, *Science* **221**, 773 (1983).
4. M. J. Kuhar and M. A. Zargin, *J. Neurochem.* **31**, 251 (1975); A. S. Horn, *Prog. Neurobiol.* **34**, 387 (1990).
5. M. C. Ritz, R. J. Lamb, S. R. Goldberg, M. J. Kuhar, *Science* **237**, 1219 (1987). Cocaine-sensitive dopamine transporters have been cloned: S. Shimada *et al.*, *ibid.* **254**, 576 (1991); J. E. Kilty, D. Lorang, S. G. Amara, *ibid.*, p. 578.
6. M. J. Kuhar, M. C. Ritz, J. W. Boja, *Trends Neurosci.* **14**, 299 (1991).
7. F. H. Gawin *et al.*, *Arch. Gen. Psychiatry* **46**, 117 (1989); M. W. Fischman, R. W. Foltin, G. Nestadt, G. D. Perlson, *J. Pharmacol. Exp. Ther.* **253**, 760 (1990).
8. K. F. Bonese, B. H. Wainer, F. W. Fitch, R. M. Rothberg, C. R. Schuster, *Nature* **252**, 708 (1974).
9. A. Tramontano, K. D. Janda, R. A. Lerner, *Science* **234**, 1566 (1986); S. J. Pollack, J. W. Jacobs, P. G. Schultz, *ibid.*, p. 1570.
10. R. A. Lerner, S. J. Benkovic, P. G. Schultz, *ibid.* **252**, 659 (1991).
11. R. D. Spealman, B. K. Madras, J. Bergman, *J. Pharmacol. Exp. Ther.* **251**, 142 (1989).
12. A. Tramontano, A. A. Ammann, R. A. Lerner, *J. Am. Chem. Soc.* **110**, 2282 (1988).
13. M. L. Bender, *Chem. Rev.* **60**, 53 (1960).
14. P. G. Schultz, *Science* **240**, 426 (1988).
15. K. D. Janda, S. J. Benkovic, D. A. McLeod, D. M. Schloeder, R. A. Lerner, *Tetrahedron* **47**, 2503 (1991).
16. K. D. Janda, M. I. Weinhouse, T. Danon, K. A. Pacelli, D. M. Schloeder, *J. Am. Chem. Soc.* **113**, 5427 (1991).
17. K. Zhao and D. W. Landry, *Tetrahedron* **49**, 363 (1993).
18. R. J. P. Corriu, F. Lanneau, D. Leclercq, *ibid.* **36**, 1617 (1980); K. D. Janda, S. J. Benkovic, R. A. Lerner, *Science* **244**, 437 (1989); S. J. Pollack, P. Hsiun, P. G. Schultz, *J. Am. Chem. Soc.* **111**, 5961 (1989); S. Ikeda, M. I. Weinhouse, K. D. Janda, R. A. Lerner, S. Danishefsky, *ibid.* **113**, 7763 (1991); I. Fujii, R. A. Lerner, K. D. Janda, *ibid.*, p. 8528.
19. Six BALB/c mice were immunized with the analog-carrier **1a** by both subcutaneous (30 μg) and intraperitoneal (120 μg) routes. Initial immunization was performed with 1:1 Freund's complete adjuvant, and boosting injections were administered with incomplete adjuvant at 2-week intervals. The animals were phlebotomized (200 μl) 14 days after the boost, and the plasma was separated from clot and stored at −78°C. In an ELISA, plastic 96-well plates were coated with ester **5** coupled to ovalbumin (4 μg per well) and incubated with dilutions of serum. Goat anti-mouse IgG coupled to horseradish peroxidase was used as the secondary antibody and indicator. Negative controls included ovalbumin without ligand, nonimmune test serum, omission of the test serum, and omission of the secondary antibody. By the third boost, several mice had developed antibody titers of 1:3000. One of these was boosted by tail-vein injection (50 μg, without adjuvant). On the fifth day after the boost, the spleen was harvested and hybridomas were prepared. Colonies positive for anti-analog antibodies by ELISA were plated to limiting dilution and subtyped (mAb 3B9 and 6A12 were both subtyped to IgG_1).
20. For each of the cell lines, 2 × 10^6 hybridoma cells were placed into a mouse peritoneum that had been pretreated with pristane. The harvested ascites were subjected to affinity chromatography on a preparative protein A HPLC column (Bio-Rad) (the purity of mAb 3B9 and 6A12 was >90% by SDS–polyacrylamide gel electrophoresis).
21. The $^{14}C_{benzoyl}$-cocaine was synthesized from methyl ecgonine and the acyl chloride of ^{14}C–benzoic acid. The specific activity was 111 μCi/mmol (Amersham).
22. The hydrolysis reaction mixture was analyzed by HPLC (Perkin-Elmer) with an analytical reverse-phase C_{18} column (VYDAC) with an acetonitrile-water (0.1% trifluoroacetic acid) gradient and the detector set at 220 nM. The methyl ester of cocaine spontaneously hydrolyzed to benzoyl ecgonine with a $t_{1/2}$ = 20 hours (pH 7). Thus, benzoyl ecgonine is not available as a benzoyl esterase substrate at the early reaction times (<1 hour) during which the detailed kinetics are studied, and the release of benzoic acid is attributed solely to cocaine hydrolysis.
23. D. J. Stewart, T. Inaba, B. Tang, M. Kalow, *Life Sci.* **20**, 1557 (1977).
24. D. W. Landry and G. X.-Q. Yang, unpublished data.
25. The $^3H_{phenyl}$-cocaine was synthesized by the catalytic hydrogenation of 4′-iododocaine with high–specific-activity tritium gas under Pd-C catalysis (New England Nuclear, Boston, MA). We obtained the 4′-iododocaine by coupling ecgonine methyl ester and 4-iodobenzoyl chloride under AgCN catalysis.
26. D. J. Stewart, T. Inaba, M. Lucassen, W. Kalow, *Clin. Pharmacol. Ther.* **25**, 464 (1978); S. J. Gatley, *Biochem. Pharmacol.* **41**, 1249 (1991).
27. One equivalent of **1b** reduced the rate of 3B9-catalyzed cocaine hydrolysis by ~50%.
28. R. A. Lerner, A. S. Kang, J. D. Bain, D. R. Burton, C. F. Barbas, *Science* **258**, 1313 (1992).
29. These concentrations are over tenfold higher than peripheral venous concentrations and correspond to estimated CNS cocaine levels; J. S. Fowler *et al.*, *Synapse* **4**, 371 (1989).

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Translocation of TCRα Chains into the Lumen of the Endoplasmic Reticulum and Their Degradation

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After synthesis, the α chain of the T cell antigen receptor (TCRα) can form a complex with other TCR chains and move to the cell surface, or TCRα can undergo degradation in the endoplasmic reticulum (ER) if it remains unassembled. The mechanism of translocation and degradation in the ER is unclear. It was found that the putative transmembrane region of TCRα ($α_{tm}$) was incompetent on its own to act as a transmembrane region. Molecules that contained $α_{tm}$ were translocated into the ER lumen and then underwent either rapid degradation or secretion, depending on the sequence of the cytoplasmic domain. A specific signal for ER degradation within $α_{tm}$ does not appear to be present.

The T cell antigen receptor TCRαβ is a disulfide-linked heterodimer and is associated with nonpolymorphic components of the CD3 molecule (γ, δ, ε, ζ, and η) at the cell surface (*1*). In this oligomeric complex TCRα probably spans the membrane by a single transmembrane peptide domain (*2*). Partially assembled or unassembled TCR and CD3 components are retained, degraded, or both in the ER or are targeted for lysosomal degradation (*3*, *4*). In cDNA-transfected COS-7 cells, murine TCRα has been reported to be rapidly degraded in a nonlysosomal compartment before entering the Golgi apparatus (ER degradation) (*5*–

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7). The transmembrane peptide of TCRα was thought to contain signals for ER degradation (7).

In general, a transmembrane peptide is an amphiphilic α helix of approximately 20 amino acids, in which preferably >11 consecutive residues are hydrophobic and confer stability to the peptide in the lipid bilayer (8). However, the suggested transmembrane peptide of TCRα contains Arg^{233}, Lys^{238}, Asn^{243}, and Thr^{247} [numbering for the L17 TCRα (2)], which interrupt the hydrophobic region four times. An additional 17.2 kcal/mol would be required to keep these sequences in the membrane as compared with a molecule whose polar residues are replaced by alanine (9). The side chains of Arg^{233} and Lys^{238} point in opposite directions (140 degrees), which also contributes to the thermodynamic undesirability of the α helix. The question, therefore, has been raised whether such a transmembrane region would by itself be stable in the lipid bilayer (10). TCRα, at least as a single polypeptide, may not be anchored to the membrane but may reside in the ER lumen. Exposure of the hydrophobic transmembrane region to the lumenal environment could cause the degradation of such molecules.

Human TCRα transfected into HeLa cells (Fig. 1A), as well as murine TCRα (6, 7), remained sensitive to endoglycosidase H (Endo H) digestion for 8 hours (that is, it was retained in the ER) and disappeared rapidly from a total cell lysate but was not secreted into the culture medium (Fig. 1B). However, truncated TCRα that had no transmembrane (TM) or cytoplasmic (CYT) regions (stop codon replaced Val^{229}) was also retained in the ER although it was not degraded rapidly (Fig. 1C); thus, the extracellular (EX) region of this truncated TCRα also has an ER retention signal or signals. The specific role of the TCRα TM region in localization was examined by construction of a chimeric molecule ($CD4_{ex}$·α_{tm20}·$CD4_{cyt}$) that contained the EX domain of CD4 [which has no ER retention signal (11)], the putative TM region of human TCRα [20 amino acids (aa)], and the CYT region of CD4 (38 aa) (Fig. 1A) and by transfecting it into HeLa cells. The $CD4_{ex}$·α_{tm20}·$CD4_{cyt}$ appeared in the culture medium 4 hours after pulse labeling, and more than half was secreted in 12 hours (Fig. 1D). The total amount of cell-associated and secreted $CD4_{ex}$·α_{tm20}·$CD4_{cyt}$ was not significantly decreased during the 12-hour chase period—that is, no rapid degradation occurred. The secreted $CD4_{ex}$·α_{tm20}·$CD4_{cyt}$ contained a glycan that was resistant to Endo H digestion (Fig. 1D) (12). Thus, $CD4_{ex}$·α_{tm20}·$CD4_{cyt}$ followed the secretory pathway after it was modified in the Golgi apparatus and was secreted into the medium.

A murine TCR α_{tm} of 23 aa (7) has been studied that includes three additional amino acids at the NH_2-terminal side (Asn-Leu-Ser) that are also in human TCRα. Thus, the α_{tm} in the chimeric molecule was extended at its NH_2-terminus to Asn^{226} or to Thr^{220} ($CD4_{ex}$·α_{tm23}·$CD4_{cyt}$ and $CD4_{ex}$·α_{tm29}·$CD4_{cyt}$, respectively) (Fig. 1A). These molecules each contain an additional potential glycosylation site. With increased chase time, the 56-kD band in the $CD4_{ex}$·α_{tm29}·$CD4_{cyt}$ gel was converted to a 58-kD band (Fig. 2A). A neuraminidase-sensitive, Endo H–resistant 63-kD protein appeared in the culture medium after 4 hours of chase, and the amount of this protein increased while the amounts of the endo H–sensitive 56-kD and 58-kD bands decreased (Fig. 2B). All three bands, however, produced the same 48-kD band after *N*-glycosidase treatment. The 63-kD protein in the culture medium was not removable by high-speed centrifugation (13). Thus, $CD4_{ex}$·α_{tm29}·$CD4_{cyt}$ is secreted into the culture medium rather than released into the culture medium either by proteolytic cleavage or by membrane fragmentation. The glycosylation patterns indicate that fully modified $CD4_{ex}$·α_{tm29}·$CD4_{cyt}$ follows the secretory pathway. $CD4_{ex}$·α_{tm23}·$CD4_{cyt}$ had essentially the same fate (13).

Translocation of such chimeric proteins to an aqueous lumenal compartment was confirmed by Na_2CO_3 buffer (pH 11.3)

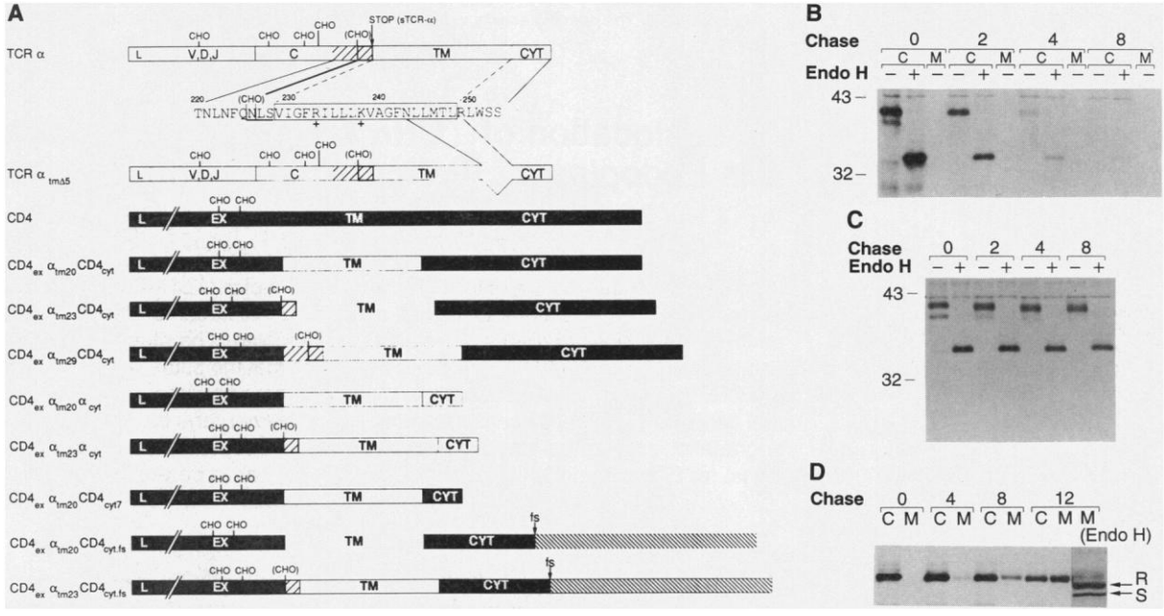

Fig. 1. Intracellular fates of TCRα, truncated TCRα, and the $CD4_{ex}$·α_{tm20}·$CD4_{cyt}$ (20). (**A**) Diagrammatic illustration of TCRα (light), CD4 (dark), and chimeric molecules composed of parts of TCRα and CD4. The TM region of the TCRα is presented with the one-letter amino acid code (15). L, leader sequence. CHO, carbohydrate residue. V, D, J, and C, L17 TCRα gene segments (2); plus (+) signs indicate positively charged residues. (**B**) ER degradation of TCRα. Immunoprecipitates of cDNA encoding TCRα-transfected and pulse chase–labeled cell lysates (C) and culture medium (M) are presented (numbers at the top indicate hours after chase). TCRα was rapidly degraded without secretion into the culture medium and was Endo H–sensitive. Molecular size standards are shown at the left in kilodaltons. (**C**) Immunoprecipitation of cell lysates shows that a TCRα without its TM and CYT regions is not degraded rapidly. TCRα found in the immunoprecipitate was all Endo H–sensitive. (**D**) Immunoprecipitation of the $CD4_{ex}$·α_{tm20}·$CD4_{cyt}$ from the cell lysate (C) or from the medium (M). Endo H–resistant (R) and Endo H–sensitive (S) forms of the secreted $CD4_{ex}$·α_{tm20}·$CD4_{cyt}$ are marked.

extraction (*14*). After samples were labeled for 30 min, ~50% of $CD4_{ex}{\cdot}\alpha_{tm29}{\cdot}CD4_{cyt}$ and trace amounts of intact CD4 were extracted with 0.1 M Na_2CO_3. After 4 hours of chase, ~80% of the $CD4_{ex}{\cdot}\alpha_{tm29}{\cdot}CD4_{cyt}$, but none of the CD4, was extracted (Fig. 2C). Thus, the α_{tm}-containing protein was translocated from the lipid bilayer to the lumenal fraction. Furthermore, whereas intact CD4 partitioned into a Triton X-114 (TX114) phase, >90% of intact TCRα partitioned into the aqueous phase (Fig. 2D) and was almost completely degraded during a 2-hour chase period (*13*). Thus, TCRα, like the chimeric molecules, also appeared to be translocated into the ER lumen rather than anchored to the ER membrane, presumably because of an intrinsic property of the TCRα TM sequences.

The two positively charged amino acids in α_{tm} have been suggested to be a specific motif for ER retention and degradation (*7*). Mutation of these two amino acids to hydrophobic residues abolished ER degradation of human TCRα (Fig. 3A). These charged amino acids were suggested to be recognized in the lipid bilayer, resulting in rapid degradation in or near the ER (*14*). If so, molecules that contain the same TM peptide should share the same fate. But $CD4_{ex}{\cdot}\alpha_{tm}{\cdot}CD4_{cyt}$, which also contains these two charged residues, is secreted, not degraded. Furthermore, $TCR\alpha_{tm\Delta5}$, in which five hydrophobic residues of α_{tm} are deleted (Fig. 1A), was degraded rapidly in the ER without secretion into the culture medium (Fig. 3B). The $TCR\alpha_{tm\Delta5}$ contains only 12 hydrophobic aa, with insertion of two charged residues (indicated in bold) in the middle of the region [VIGF**R**ILLL**K**VAGF**NR** (*15*)]; it has no features of a TM peptide and is expected to be present in the ER lumen, rather than in the ER membrane. This result also suggests that recognition for ER degradation did not take place in the lipid bilayer but in the lumen of the ER and that the mutations of the charged residues in α_{tm} stabilized TCRα in the lipid bilayer.

One of the major structural differences between molecules that are rapidly degraded [membrane immunoglobulin M (IgM) (*16*), TCRα, and Tac-TCRα chimeras (*7*)] and those secreted without degradation ($CD4_{ex}{\cdot}\alpha_{tm}{\cdot}CD4_{cyt}$) is the length of their CYT regions (3, 5, or 10 aa versus 40 aa). The CYT regions of the $CD4{\cdot}\alpha_{tm}{\cdot}CD4$ chimeras were shortened either by replacement with the 5-aa CYT region of TCRα ($CD4_{ex}{\cdot}\alpha_{tm}{\cdot}\alpha_{cyt}$) (Fig. 1A) or by the mutation of Gln^{403} of the CD4 CYT region to a stop codon, leaving 7 aa in the CYT region ($CD4_{ex}{\cdot}\alpha_{tm20}{\cdot}CD4_{cyt7}$) (Fig. 1A). These chimeric molecules were degraded rapidly (Fig. 3, C through E).

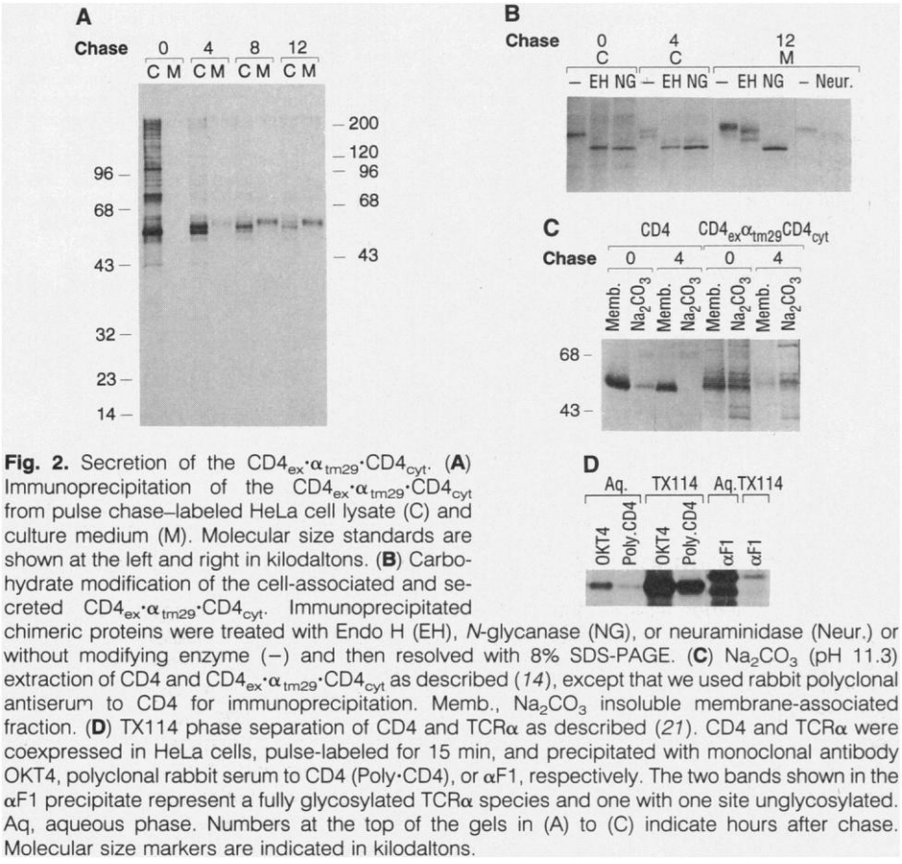

Fig. 2. Secretion of the $CD4_{ex}{\cdot}\alpha_{tm29}{\cdot}CD4_{cyt}$. (**A**) Immunoprecipitation of the $CD4_{ex}{\cdot}\alpha_{tm29}{\cdot}CD4_{cyt}$ from pulse chase–labeled HeLa cell lysate (C) and culture medium (M). Molecular size standards are shown at the left and right in kilodaltons. (**B**) Carbohydrate modification of the cell-associated and secreted $CD4_{ex}{\cdot}\alpha_{tm29}{\cdot}CD4_{cyt}$. Immunoprecipitated chimeric proteins were treated with Endo H (EH), *N*-glycanase (NG), or neuraminidase (Neur.) or without modifying enzyme (–) and then resolved with 8% SDS-PAGE. (**C**) Na_2CO_3 (pH 11.3) extraction of CD4 and $CD4_{ex}{\cdot}\alpha_{tm29}{\cdot}CD4_{cyt}$ as described (*14*), except that we used rabbit polyclonal antiserum to CD4 for immunoprecipitation. Memb., Na_2CO_3 insoluble membrane-associated fraction. (**D**) TX114 phase separation of CD4 and TCRα as described (*21*). CD4 and TCRα were coexpressed in HeLa cells, pulse-labeled for 15 min, and precipitated with monoclonal antibody OKT4, polyclonal rabbit serum to CD4 (Poly·CD4), or αF1, respectively. The two bands shown in the αF1 precipitate represent a fully glycosylated TCRα species and one with one site unglycosylated. Aq, aqueous phase. Numbers at the top of the gels in (A) to (C) indicate hours after chase. Molecular size markers are indicated in kilodaltons.

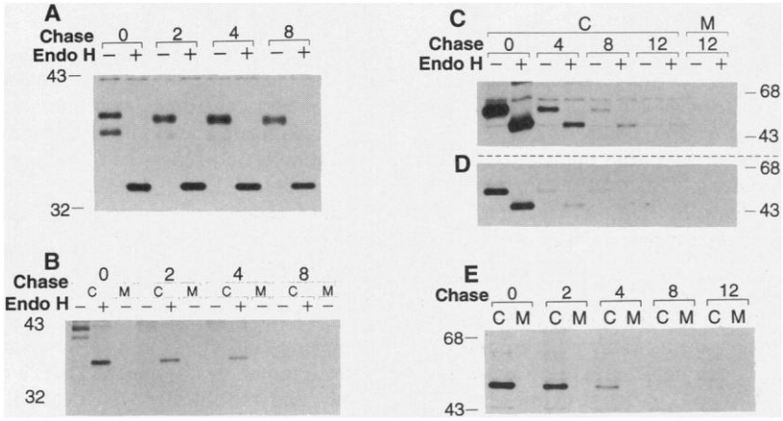

Fig. 3. ER degradation of chimeric proteins with short cytoplasmic tails. (**A**) Mutation of two charged amino acids (Arg and Lys) in the TM region of the TCRα into hydrophobic residues (Leu and Ile) prevents rapid ER degradation of the TCRα. (**B**) Deletion of five hydrophobic amino acids from the TM region ($TCR\alpha_{tm\Delta5}$) does not have much effect on the rapid degradation of the TCRα chain. Rapid ER degradation and Endo H sensitivity of the (**C**) $CD4_{ex}{\cdot}\alpha_{tm20}{\cdot}\alpha_{cyt}$, (**D**) $CD4_{ex}{\cdot}\alpha_{tm23}{\cdot}\alpha_{cyt}$, and (**E**) $CD4_{ex}{\cdot}\alpha_{tm20}{\cdot}CD4_{cyt7}$. Numbers at the top of the gels indicate hours after chase; C and M are as in Fig. 2. Molecular size markers are indicated in kilodaltons.

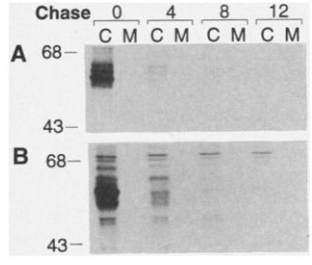

Fig. 4. ER degradation of frame-shift mutants (fs). (**A**) $CD4_{ex}{\cdot}\alpha_{tm23}{\cdot}CD4_{cyt.fs}$ and (**B**) $CD4_{ex}{\cdot}\alpha_{tm20}{\cdot}CD4_{cyt.fs}$. These cell-associated chimeric proteins were all Endo H–sensitive. Molecular size markers are indicated in kilodaltons; C and M are as in Fig. 2.

Thus, the determinant of escape from rapid ER degradation may be proper folding of the hydrophobic TM peptide in the lumen of the ER, driven by the CYT region. The 40-aa-long CYT region of CD4, predicted to have a highly ordered structure, including an NH_2-terminal α-helical region (*17*), may induce proper folding of α_{tm}. To explore this possibility, we replaced the CYT region of $CD4_{ex}$·α_{tm}·$CD4_{cyt}$ with a 67-aa-long peptide that contains the intact NH_2-terminal 20 aa of the $CD4_{cyt}$, followed by 47 random aa created by a frame-shift mutation at Gln^{421} of the CD4 cDNA ($CD4_{cyt.fs}$) (Fig. 1A). CD4 with this CYT region peptide was not susceptible to phosphorylation-dependent down-modulation, probably because of disrupted conformation in the first half of $CD4_{cyt}$ (*18*). $CD4_{ex}$·α_{tm}·$CD4_{cyt.fs}$ was rapidly degraded without secretion (Fig. 4). Thus, whether the chimeric molecules were secreted or degraded was determined by the structure of the COOH-terminal cytoplasmic tail rather than by the length of the tail.

Thus, the α_{tm} contributes to translocation of TCRα into the ER lumen and to recognition by a lumenal degradative system. It is apparently incompetent to function as a TM region in the absence of association with other polypeptides. With a short CYT region, the α_{tm} may not fold properly in the lumen of the ER. Under these circumstances, TCRα would expose its hydrophobic TM region to the lumenal environment, resulting in rapid degradation. A putative soluble T cell suppressor factor is reported to contain TCRα in association with a second subunit (*19*). The structure of α_{tm} could have an important physiological consequence through the secretion of such a heterodimer.

REFERENCES AND NOTES

1. H. Clevers, B. Alarcon, T. Wileman, C. Terhorst, *Annu. Rev. Immunol.* **6**, 629 (1988).
2. J. M. Leiden, J. D. Fraser, J. L. Strominger, *Immunogenetics* **24**, 17 (1986).
3. B. Alarcon, B. Berkhout, J. Breitmeyer, C. Terhorst, *J. Biol. Chem.* **263**, 2953 (1988).
4. J. Lippincott-Schwartz, J. S. Bonifacino, L. C. Yuan, R. D. Klausner, *Cell* **54**, 209 (1988).
5. T. Wileman, G. R. Carson, M. Concino, A. Ahmed, C. Terhorst, *J. Cell Biol.* **110**, 973 (1990).
6. J. Lippincott-Schwartz *et al.*, *Cell* **60**, 821 (1990).
7. J. S. Bonifacino, C. K. Suzuki, R. D. Klausner, *Science* **247**, 79 (1990); M. Baniyash, P. Garcias-Morales, J. S. Bonifacio, L. E. Samelson, R. D. Klausner, *J. Biol. Chem.* **263**, 9874 (1988); J. S. Bonifacino, P. Cosson, R. D. Klausner, *Cell* **63**, 503 (1990); N. Manolios, J. S. Bonifacino, R. D. Klausner, *Science* **249**, 274 (1990).
8. D. Eisenberg, E. Schwarz, M. Komaromy, R. Wall, *J. Mol. Biol.* **179**, 125 (1984).
9. T. E. Creighton, *Proteins* (Freeman, New York, 1984), p. 142.
10. J. Goverman *et al.*, *Cell* **60**, 929 (1990).
11. J. Shin, R. L. Dunbrack, Jr., S. Lee, J. L. Strominger, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 1918 (1991).
12. CD4 itself has two glycans, one complex and the other high mannose (*11*). The band marked R in Fig. 1D is the mature molecule from which one Endo H–sensitive glycan has been removed.
13. J. Shin, unpublished observations.
14. J. S. Bonifacino, P. Cosson, N. Shah, R. D. Klausner, *EMBO J.* **10**, 2783 (1991).
15. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
16. R. Sitia *et al.*, *Cell* **60**, 781 (1990); R. Sitia, M. S. Neuberger, C. Milstein, *EMBO J.* **6**, 3969 (1987).
17. J. Shin, R. L. Dunbrack, Jr., S. Lee, J. L. Strominger, *J. Biol. Chem.* **266**, 10658 (1991).
18. J. Shin, C. Doyle, Z. Yang, D. Kappes, J. L. Strominger, *EMBO J.* **9**, 425 (1990).
19. V. K. Kuchroo *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8700 (1991).
20. We made CD4-TCRα chimeric cDNA by using a mutant CD4 cDNA that has unique Nco I and Spe I sites at the 5′- and 3′-ends of the TM coding region. The TM region of CD4 was replaced with double-stranded DNA encoding the α_{tm}, and the Spe I site was remutated to the original CD4 sequence. To construct the $TCR\alpha_{tm\Delta5}$, we cut the TCRα cDNA at the Bgl II site at the 3′-end and at the Nci I site at the middle of the TM region and ligated the cDNA with double-stranded DNA encoding Gly-Phe-Asn-Arg-Leu-Trp-Ser-Ser-stop. After transfection of the cDNA in the expression vector pcDNAI (Invitrogen) into HeLa cells by the calcium phosphate method [C. Chen and H. Okayama, *Mol. Cell. Biol.* **7**, 2745 (1987)], cells were labeled with [^{35}S]methionine (200 μCi/ml) for 15 min and chased in complete medium for 0 to 12 hours (*11*). We used monoclonal antibodies αF1 and Leu3a or OKT4 to immunoprecipitate TCRα and CD4-TCRα chimeric proteins, respectively [R. S. Blumberg *et al.*, *J. Biol. Chem.* **265**, 14036 (1990); (*18*)]. We solubilized immunoprecipitates for enzyme digestion by boiling them in 0.5% SDS in 10 mM sodium phosphate. Samples were dissolved in 2 volumes of 10 mM sodium phosphate buffer (pH 7.0) for Endo H or neuraminidase digestion or dissolved in 10 volumes of 0.5% octylglucoside in 10 mM sodium phosphate (pH 7.0) for *N*-glycosidase digestion. Samples were resolved by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by fluorography.
21. C. Bordier, *J. Biol. Chem.* **256**, 1604 (1981).

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Germ Line Transmission of a Yeast Artificial Chromosome Spanning the Murine α_1(I) Collagen Locus

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Molecular complementation of mutant phenotypes by transgenic technology is a potentially important tool for gene identification. A technology was developed that allows the transfer of a physically intact yeast artificial chromosome (YAC) into the germ line of the mouse. A purified 150-kilobase YAC encompassing the murine gene *Col1a1* was efficiently introduced into embryonic stem (ES) cells via lipofection. Chimeric founder mice were derived from two transfected ES cell clones. These chimeras transmitted the full length transgene through the germ line, generating two transgenic mouse strains. Transgene expression was visualized as nascent transcripts in interphase nuclei and quantitated by ribonuclease protection analysis. Both assays indicated that the transgene was expressed at levels comparable to the endogenous collagen gene.

The size of many mammalian genes such as *NF-1*, *MDX*, or the gene encoding factor VIII is in the megabase range (*1–4*); this size range exceeds the limits of conventional cloning techniques, thus impeding the functional analysis of such genes in transgenic model systems. In addition to facilitating the study of known genes, the ability to genetically manipulate large pieces of DNA in mice would be useful for the identification of genes that have been localized by genetic and physical means to certain chromosomal regions. For example, if it were possible to transfer YACs (*5*) to the germ line of mutant mice, molecular complementation of the mutant phenotype would functionally identify genes or gene complexes present on the YAC, allowing the positional cloning and validation of new genes. Toward this goal, we have established procedures that permit the efficient transfer of physically intact YAC DNA into ES cells and we report the transmission of a gene locus carried on a 150-kb DNA fragment through the germ line.

As a model for establishing an in vivo complementation system, we have used the Mov13 mouse strain, which carries a molecularly and biologically well defined mutation. This strain was generated by random insertional mutagenesis after retroviral infection of early mouse embryos. A provirus had integrated into the gene for α_1 (I) collagen (*Col1a1*), interfering with gene expression and resulting in an embryonic recessive lethal phenotype at day 13 of gestation (*6*).

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