precursor (*13*) modified in its p12 region (7), transforms cells is unknown. Since the final target cell population is clonal, the expression of the defective viral genome does not seem sufficient by itself to transform these cells and, most likely, another genetic event is required for their full transformation. Therefore, the initial causal disease appears to be a benign (hyperplasia or dysplasia) or a malignant neoplasia leading to a secondary immunodeficiency state. The infected target cells could be induced to secrete one or more factors detrimental to the immune system, directly or indirectly, or they could stop secreting factors essential for the normal function of the immune system. Alternatively, their interaction with other cells of the immune system could trigger the immunodeficiency.

AIDS patients carry few infected cells and show little virus replication (*21, 22*), a status that appears similar to the one seen in these mice infected with helper-free stocks of defective virus. Our results emphasize the need to determine whether virus replication is necessary for the progression of AIDS and whether this human syndrome is also the consequence of a neoplasia. If the primary defect in AIDS is a neoplasia, as it appears to be in this murine model, cofactors would be expected to play an important role in the initiation of the disease. One of the practical implications would be the immediate availability of several anticancer drugs to try to prevent the development of AIDS and to treat it.

REFERENCES AND NOTES

1. R. Latarget and J. F. Duplan, *Int. J. Radiat. Biol.* **5**, 339 (1962).
2. E. Legrand, R. Daculsi, J. F. Duplan, *Leuk. Res.* **5**, 223 (1981).
3. D. E. Mosier, R. A. Yetter, H. C. Morse III, *J. Exp. Med.* **161**, 766 (1985).
4. D. E. Mosier, *Immunol. Invest.* **15**, 233 (1986).
5. S. P. Klinken, T. N. Fredrickson, J. W. Hartley, R. A. Yetter, H. C. Morse III, *J. Immunol.* **140**, 1123 (1988).
6. R. M. L. Buller, R. A. Yetter, T. N. Fredrickson, H. C. Morse III, *J. Virol.* **61**, 383 (1987).
7. D. C. Aziz, Z. Hanna, P. Jolicoeur, *Nature* **338**, 505 (1989).
8. R. Mann, R. C. Mulligan, D. Baltimore, *Cell* **33**, 153 (1983).
9. One infectious helper MuLV per milliliter can be detected by this assay.
10. L. Des Groseillers, E. Rassart, P. Jolicoeur, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4203 (1983). This assay can detect one infected cell per 10^8 cells.
11. J. A. Levy, *Curr. Top. Microbiol. Immunol.* **79**, 111 (1978).
12. C. Simard and P. Jolicoeur, unpublished observations.
13. M. Huang and P. Jolicoeur, unpublished observations.
14. S. K. Chattopadhyay, M. R. Lander, E. Rands, D. R. Lowy, *Proc. Natl. Acad. Sci. U.S.A.* 77, 5774 (1980).
15. In situ hybridization was performed as described [B. Hogan, F. Costantini, E. Lacy, Eds., *Manipulating the Mouse Embryo* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986)] with ^{35}S-labeled D30 DNA probe. Cells from lymph nodes were dispersed in phosphate-buffered saline containing 10% calf serum and attached to poly-L-lysine–treated slides by cytospin. After autoradiography (at 4°C for 5 days), the slides were stained with hematoxylin and eosin. Cells with 20 grains or more were considered positive. Typically, negative cells had less than 2 grains.
16. The sensitivity of the assay allowed the detection of 0.02 to 0.1 proviral copy per cell.
17. L. Wolff and S. Ruscetti, *Science* **228**, 1549 (1985).
18. P. L. Green, D. A. Kaehler, R. Risser, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5932 (1987).
19. Y. Poirier and P. Jolicoeur, *J. Virol.* **63**, 2088 (1989).
20. P. K. Pattengale *et al.*, *Am. J. Pathol.* **107**, 362 (1982).
21. F. Wong-Staal and R. C. Gallo, *Nature* **317**, 395 (1985).
22. A. S. Fauci, *Science* **239**, 617 (1988).
23. P. Chomczynski and N. Sacchi, *Anal. Biochem.* **162**, 156 (1987).
24. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
25. P. Jolicoeur and D. Baltimore, *Cell* 7, 33 (1976).

26. We thank D. Kay for help in setting up the in situ hybridization procedure. Supported by grants (to P.J.) from the Medical Research Council of Canada and from the National Cancer Institute of Canada.

31 July 1989; accepted 6 October 1989

Cyclosporin A Specifically Inhibits Function of Nuclear Proteins Involved in T Cell Activation

ELIZABETH A. EMMEL, CORNELIS L. VERWEIJ, DAVID B. DURAND, KAY M. HIGGINS, ELIZABETH LACY, GERALD R. CRABTREE

One action of cyclosporin A thought to be central to many of its immunosuppressive effects is its ability to inhibit the early events of T lymphocyte activation such as lymphokine gene transcription in response to signals initiated at the antigen receptor. Cyclosporin A was found to specifically inhibit the appearance of DNA binding activity of NF-AT, AP-3, and to a lesser extent NF-κB, nuclear proteins that appear to be important in the transcriptional activation of the genes for interleukin-2 and its receptor, as well as several other lymphokines. In addition, cyclosporin A abolished the ability of the NF-AT binding site to activate a linked promoter in transfected mitogen-stimulated T lymphocytes and in lymphocytes from transgenic mice. These results indicate that cyclosporin A either directly inhibits the function of nuclear proteins critical to T lymphocyte activation or inhibits the action of a more proximal member of the signal transmission cascade leading from the antigen receptor to the nucleus.

CYCLOSPORIN A (CsA) IS A CYCLIC undecapeptide with highly specific immunosuppressive effects that have been useful in treating rejection of allogenic transplants. Although the mechanism of action of this drug is unknown, it appears to act early to inhibit activation of T lymphocytes by antigen (*1*). These early effects on T cells give rise to various secondary effects on B cells, macrophages, and other cells dependent on the products of activated T cells, although CsA probably also has direct effects on cells other than T lymphocytes. Cyclosporin A does not inhibit the immediate membrane events associated with triggering the antigen receptor such as inositol 1,4,5-trisphosphate (IP_3) generation or calcium mobilization (*1, 2*). However, the transcriptional activation of lymphokine genes such as interleukin-2 (IL-2), IL-4, and gamma interferon (IFN-γ) are blocked (*3*). These results suggest that CsA interferes with the function of molecules that transmit signals between membrane events such as IP_3 generation and the activation of genes in the nucleus.

Several nuclear proteins have been implicated in the regulation of the early T cell activation genes (Fig. 1A). Activator protein 1 (AP-1), NF-κB, Oct-1, and AP-3 bind to sequences within the IL-2 or IL-2 receptor promoters essential for their transcriptional activation (*4–8*). A protein called "nuclear factor of activated T cells" (NF-AT) binds to sequences within the IL-2 enhancer necessary for both inducibility and T cell–specific expression. NF-AT appears to be the product of an earlier gene in the T cell activation pathway and probably accounts for the requirement for protein synthesis for IL-2 gene activation (*5*).

We initially used the human Jurkat T cell line to determine whether CsA alters the binding or function of these nuclear proteins because Jurkat cells mimic many of the early events of T cell activation (*9*). Both IL-2 and IL-2 receptor genes are activated by triggering the antigen receptor of Jurkat cells and, as with resting peripheral T cells, the activation of the IL-2, IL-4, and IFN-γ genes is inhibited by CsA (*3, 10, 11*), whereas the IL-2 receptor gene is relatively unaffected (*12*).

E. A. Emmel, C. L. Verweij, D. B. Durand, G. R. Crabtree, The Howard Hughes Medical Institute at Stanford University, Stanford, CA 94305.
K. M. Higgins and E. Lacy, Molecular Biology Program, Sloan-Kettering Memorial Institute, New York, NY 10021.

We tested the effect of CsA on a series of internal deletion mutations of the IL-2 enhancer in directing transcription of the chloramphenicol acetyltransferase (CAT) gene. The deletion mutants were transfected into Jurkat cells along with an internal control for transfection efficiency, and the cells were stimulated with phytohemagglutinin (PHA) and phorbol 12-myristate 13-acetate (PMA) in the presence or absence of CsA (*4*). Mutation of the NF-AT site, the Oct-1 site, or the AP-1 site reduced activity of the enhancer (Fig. 1B) as reported (*4*). No single deletion eliminated inhibition by CsA, indicating that more than a single region is responsible for the inhibitory effects of CsA. However, the deletion mutant −279 to −263 showed only 62% inhibition by CsA, compared to 86 to 99% inhibition with the other deletion mutants, suggesting that this site contributes to CsA action but does not totally account for it. This site corresponds to antigen receptor response element 2 (ARRE 2) previously described (*4, 5*) and is the binding site for NF-AT.

To further define the effects of CsA on the function of the IL-2 enhancer, we examined the ability of CsA to inhibit expression directed by the NF-κB binding site (*7*), by three copies of the AP-1 binding site (*13*), by three copies of the NF-AT binding site (*4*), by the wild-type IL-2 enhancer and promoter (*14*), or by the SV40 promoter (pSV2CAT), all directing the transcription of the CAT gene (Fig. 2). Cells were stimulated for 8 hours in the presence or absence of CsA at concentrations of 10 to 1000 ng/ml. Although CsA at 10 ng/ml virtually eliminated the ability of the IL-2 enhancer to activate expression of the CAT gene, it had no effect on expression directed by the SV40 promoter or the AP-1 site (Fig. 2). CsA abolished the ability of tandem repeats of the NF-AT construct to direct transcription of the CAT gene. Dependence on CsA concentration was similar to that required for the effect of CsA on the wild-type IL-2 enhancer and similar to that required for the effects of CsA on T cell activation (*1*). Smaller inhibitory effects of CsA were detected for the ability of the NF-κB site to activate expression of CAT; however, significant inhibition was not seen at concentrations that fully inhibit T cell activation, suggesting that the effects on NF-κB may not be related to the mechanism of action of the drug.

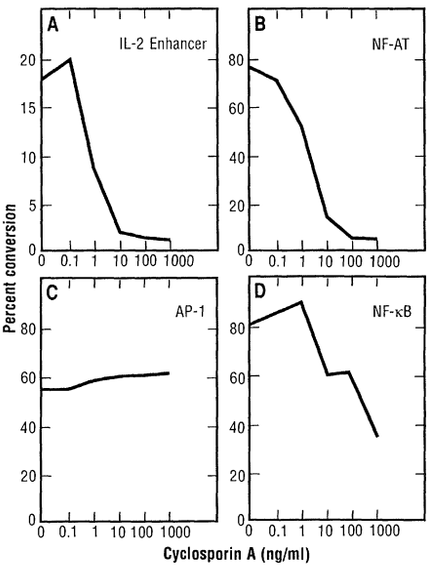

Fig. 2. CsA selectively inhibits the ability of tandem repeats of binding sites for NF-AT to activate an unrelated promoter. Expression vectors containing IL-2, NF-AT, AP-1, NF-κB, and SV40 regulatory sequences were transfected into Jurkat cells and tested for their ability to activate expression of the CAT gene after stimulation with and without CsA. The plasmids tested contained the following regulatory sequences directing transcription of the CAT gene: (**A**) IL-2 enhancer, 5′ IL-2 sequences between +47 and −574 (*14*); (**B**) NF-AT, three copies of the NF-AT binding site containing 5′ IL-2 sequence −255 to −285 linked to the gamma fibrinogen promoter (*4*); (**C**) AP-1, three copies of the AP-1 site linked to the SV40 promoter (*22*); (**D**) NF-κB, one copy of the NF-κB site from the HIV-LTR linked to the thymidine kinase promoter (*17*). These constructs were cotransfected into Jurkat cells. After a 40-hour incubation, the cells were stimulated for 8 hours with PHA (2 μg/ml), PMA (50 ng/ml), and the CsA concentrations indicated. The cells were then harvested, extracts were prepared, and CAT and luciferase were assayed (*4*). Each dose-response study was performed two to three times for each plasmid. CsA at 100 ng/ml did not affect the ability of the SV40 promoter (pSV2CAT) or the RSV promoter to direct expression of the CAT gene.

To examine the effect of CsA on the binding of the proteins believed to be involved in the regulation of IL-2 and other early T cell activation genes, we prepared nuclear extracts from Jurkat cells stimulated in the presence of CsA at concentrations from 1 to 1000 ng/ml (Fig. 3). End-labeled oligonucleotides of the AP-1, AP-3, NF-κB, and NF-AT binding sites were used to assay the respective proteins. AP-1 binding was unaffected by treating the cells with CsA at any of the concentrations tested. NF-κB binding was reduced 10 to 20% in nuclear extracts of CsA-treated cells. The binding of AP-3 was reduced 60% and NF-AT was reduced 85 to 95% by CsA at 10 ng/ml (Fig. 3). These effects on NF-AT and AP-3 correlate well with the concentration of CsA that inhibits T cell activation (*1*). No effect of CsA on DNA binding of any of these proteins was observed when CsA was added directly to the binding reaction, indicating that CsA blocks physiologic processes essential for the appearance of activity.

To determine whether the marked inhibitory effects mediated through the NF-AT site were physiologically significant in the whole animal, we prepared transgenic mice with a construct containing three copies of the NF-AT site directing transcription of the SV40 T antigen gene (*15*). Activation of spleen cells from these animals with ionomycin and PMA stimulated properly initiated transcription from the integrated transgene

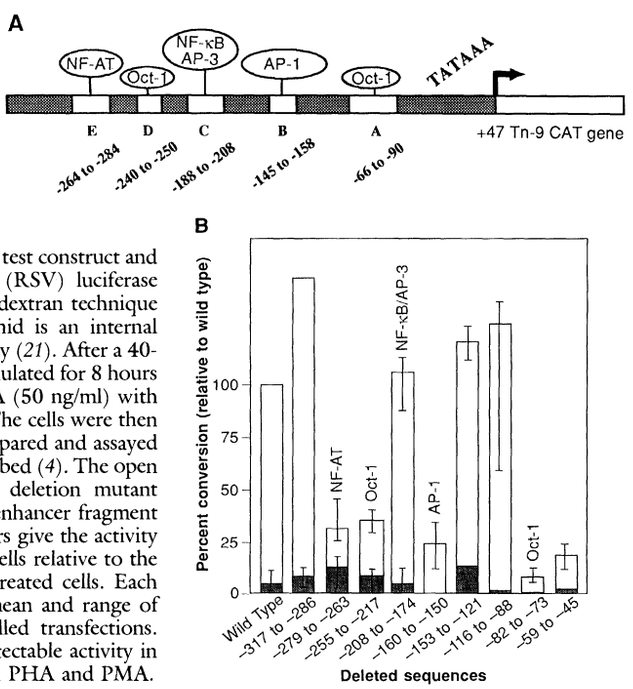

Fig. 1. No single deletion of the IL-2 enhancer abolishes CsA sensitivity. (**A**) The IL-2 enhancer with known protein binding sites. (**B**) The effect of CsA on the ability of deletion mutations of the IL-2 enhancer to activate transcription of the CAT gene (*4*). Jurkat cells were cotransfected with 20 μg of the test construct and 2 μg of Rous sarcoma virus (RSV) luciferase DNA with a modified DEAE-dextran technique (*4*). The RSV luciferase plasmid is an internal control for transfection efficiency (*21*). After a 40-hour incubation, cells were stimulated for 8 hours with PHA (2 μg/ml) and PMA (50 ng/ml) with and without CsA (10 ng/ml). The cells were then harvested and extracts were prepared and assayed for CAT and luciferase as described (*4*). The open bars give the activity of the deletion mutant relative to the wild-type IL-2 enhancer fragment −319 to +47. The stippled bars give the activity of the mutant in CsA-treated cells relative to the activity of the mutant in nontreated cells. Each bar and error represents the mean and range of two to four internally controlled transfections. None of the constructs had detectable activity in the absence of stimulation with PHA and PMA.

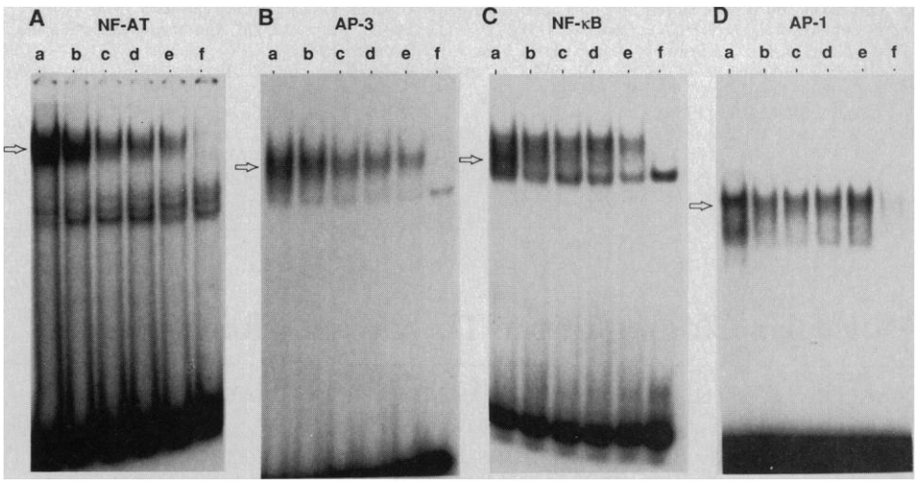

Fig. 3. The effects of CsA on the appearance of binding activity of NF-AT, AP-3, NF-κB, and AP-1. Jurkat cells were stimulated with PHA (2 μg/ml) and PMA (50 ng/ml) in the presence of CsA at the following concentrations for 8 hours: lane a, none; lane b, 1 ng/ml; lane c, 10 ng/ml; lane d, 100 ng/ml; lane e, 1000 ng/ml; lane f, nonstimulated. The cells were then harvested and nuclear extracts were prepared (*22*). The resulting extracts were desalted by centrifugation (80*g*) through a 3-ml P6DG column (Bio-Rad) at 4°C and protein concentrations were determined by Bradford assay (Bio-Rad). Extracted protein (10 μg) was then incubated for 60 min with the probes indicated below as described in (*4*), with the exception of the AP-1 oligonucleotide, which was incubated in the following buffer: 50 m*M* tris (*p*H 7.9), 13 m*M* $MgCl_2$, 1 m*M* EDTA, 1 m*M* dithiothreitol, and 20% glycerol. Protein-DNA complexes were separated from free oligonucleotide on low ionic strength 5% polyacrylamide gels (*23*). The following end-labeled probes were used: (**A**) The NF-AT binding site from the −254 to −285 region of the IL-2 enhancer, 5′ GGAGGAAAAACTGTTTCATACAGAAGGCGT (*4*); (**B**) the AP-3 binding site from the SV40 enhancer used for the affinity purification of AP-3, 5′ GATCTGTGGAAAGTCCCA (*25*); (**C**) the NF-κB binding site from the kappa light chain enhancer, 5′ GATCTCAGAGGGGACTTTCCGAG (*24*); (**D**) the AP-1 site from SV40, 5′ GTGACTCAGCGCG (*13*). Specific DNA-protein complexes corresponding to each protein are indicated with arrows. The NF-κB complex runs as a broad band and corresponds to the induced band indicated by the arrow.

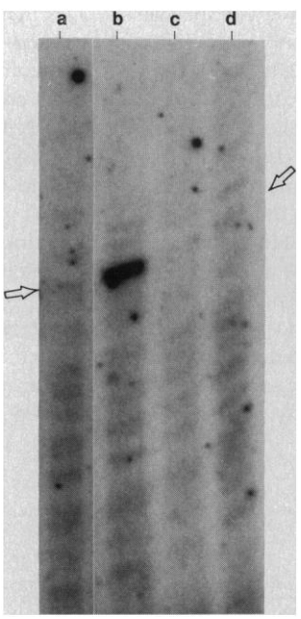

Fig. 4. CsA inhibits the ability of tandem repeats of the NF-AT site to direct transcription of the SV40 T antigen gene in transgenic mice. The construct used to prepare the transgenic animals consists of four copies of the NF-AT site inserted at position −80 of the IL-2 promoter, which is in turn fused to the SV40 T antigen at +47 (*15*). The IL-2 promoter is inactive in transgenic mice without the NF-AT site (*15*). Splenic lymphocytes were stimulated as described above for 2 hours in the presence or absence of CsA. RNA was prepared and correctly initiated transcripts from the transgene quantitated by ribonuclease protection (*26*). Properly initiated transcripts have a size of 47 bases. The distortion of the lanes is caused by an electrophoretic artifact, and the correct migration of 47 bases of the IL-2 T antigen transcript is indicated with arrows. Results similar to these were found when spleen cells were stimulated with antibody to CD3 (*15*). Lane a, nonstimulated; lane b, ionomycin + PMA; lane b, ionomycin + PMA; lane c, ionomycin + PMA + CsA; and lane d, transfer RNA.

(Fig. 4). In constructs lacking an NF-AT site, no transcriptional activation can be detected (*15*). Cyclosporin A at 100 ng/ml totally blocked the ability of ionomycin and PMA to activate NF-AT–dependent transcription of the transgene. Since transcription is dependent on the NF-AT site in these mice (*15*), these results indicate that the inhibitory effects of CsA on NF-AT–dependent activation occur in normal murine lymphocytes.

Although the inhibitory effects on the binding and function of the NF-AT site are of functional significance, the smaller effects of CsA on NF-κB binding and on NF-κB function at high concentrations of CsA (Fig. 2) may not be related to the mechanism of action of this drug, since the CsA concentration dependence of inhibition of the appearance of NF-κB binding does not correlate well with the CsA concentration dependence of T cell activation (*1*). Furthermore, the IL-2 receptor α promoter has been reported to contain a functional binding site for NF-κB (*16*); however, IL-2 receptor α gene activation is not inhibited by CsA (*12*). A binding site for NF-κB is present in the long terminal repeat of the human immunodeficiency virus (HIV-LTR) (*17*), yet CsA sensitivity of the HIV-LTR is independent of this site (*18*).

Peptidyl-prolyl cis-trans isomerase (PPIase), which is essential for refolding of certain proteins, is identical to cyclophilin, an intracellular binding molecule for CsA (*19, 20*). Since binding of CsA inhibits PPIase activity (*19*), CsA appears to exert its effects by inhibiting the isomerase-dependent refolding of proteins. A hypothesis that combines the PPIase results and our results is that NF-AT and AP-3 (or a protein necessary for their activity) require refolding for DNA binding or transcriptional activation, or both, and that CsA inhibition of PPIase activity prevents these proteins from assuming full activity. Such a hypothesis can be tested once NF-AT–dependent transcription in vitro is established.

REFERENCES AND NOTES

1. J. F. Borel, C. Feurer, H. U. Gubler, H. Stahelin, *Agents Actions* **6**, 468 (1976); E. M. Shevach, *Annu. Rev. Immunol.* **3**, 397 (1985); M. K. Bijsterbosch and G. G. Klaus, *Immunology* **56**, 435 (1985); N. Kumagai, S. H. Benedict, G. B. Mills, E. W. Gelfand, *J. Immunol.* **141**, 3647 (1988); J. F. Borel, C. Feurer, C. Magnee, H. Stahelin, *Immunology* **32**, 1017 (1977).
2. N. Isakov, M. I. Mally, W. Scholz, A. Altman, *Immunol. Rev.* **95**, 89 (1987); R. Wiskocil, A. Weiss, J. Imboden, R. Kamin-Lewis, J. Stobo, *J. Immunol.* **134**, 1499 (1985).
3. J. F. Elliott *et al.*, *Science* **226**, 1439 (1984); M. Bickel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3274 (1987); N. Arai *et al.*, *J. Immunol.* **142**, 274 (1989); M. Kronke *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5214 (1984).
4. G. H. Reem, L. A. Cook, J. Vilček, *Science* **221**, 63 (1983).
4. D. B. Durand *et al.*, *Mol. Cell. Biol.* **8**, 1715 (1988).
5. J.-P. Shaw *et al.*, *Science* **241**, 202 (1988); M. W. Brunvand, A. Schmidt, U. Siebenlist, *J. Biol. Chem.* **263**, 18904 (1988).
6. E. Serfling *et al.*, *EMBO J.* **8**, 465 (1989); T. M. Williams *et al.*, *J. Immunol.* **141**, 662 (1988).
7. M. J. Lenardo, A. Kuang, A. Gifford, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8825 (1988); H. Shibuya, M. Yoneyama, T. Taniguchi, *Intern. Immunol.* **1**, 43 (1989); B. Hoyos, D. W. Ballard, E. Böhnlein, M. Siekevitz, W. C. Greene, *Science* **244**, 457 (1989).
8. K. S. Ullman, C. A. Edwards, G. R. Crabtree, in preparation.
9. S. Gillis and J. Watson, *J. Exp. Med.* **152**, 1709 (1980); A. Weiss, R. L. Wiskocil, J. D. Stobo, *J. Immunol.* **133**, 123 (1984).
10. N. Arai *et al.*, *J. Immunol.* **142**, 274 (1989).
11. A. Granelli-Piperno, L. Andrus, R. M. Steinman, *J. Exp. Med.* **163**, 922 (1986).
12. T. Miyawaki, *J. Immunol.* **130**, 2737 (1983).
13. W. Lee, P. Mitchell, R. Tjian, *Cell* **49**, 741 (1987); P. Angel *et al.*, *ibid.*, p. 729.
14. T. Fujita *et al.*, *ibid.* **46**, 401 (1986); U. Siebenlist *et al.*, *Mol. Cell Biol.* **6**, 3042 (1986).

15. C. Verweij and G. R. Crabtree, in preparation.
16. E. Böhnlein *et al.*, *J. Biol. Chem.* **264**, 8475 (1989).
17. G. Nabel and D. Baltimore, *Nature* **326**, 711 (1987); E. Böhnlein *et al.*, *Cell* **53**, 827 (1988).
18. S. E. Tong-Starkesen, P. A. Luciw, B. M. Peterlin, *J. Immunol.* **142**, 702 (1989).
19. N. Takahashi, T. Hayano, M. Suzuki, *Nature* **337**, 473 (1989).
20. G. Fischer *et al.*, *ibid.*, p. 476.
21. J. R. de Wet *et al.*, *Mol. Cell. Biol.* **7**, 725 (1987).
22. G. Courtois *et al.*, *Science* **238**, 688 (1987).
23. M. G. Fried and D. M. Crothers, *Nucleic Acids Res.* **9**, 6505 (1981).
24. F. Mercurio and M. Karin, *EMBO J.* **8**, 1455 (1989); R. Chiu *et al.*, *Nature* **329**, 648 (1987).
25. R. Sen and D. Baltimore, *Cell* **47**, 921 (1986).
26. D. A. Melton *et al.*, *Nucleic Acids Res.* **12**, 7035 (1984); E. Evans, G. M. Courtois, P. L. Kilian, G. M. Fuller, G. R. Crabtree, *J. Biol. Chem.* **262**, 10850 (1987).
27. We thank J. Oberlindacher for preparation of the manuscript and A. Krensky and M. Davis for thoughtful reviews. Supported by the Howard Hughes Medical Institute and by NIH grants CA 39612 and HL 33942 to G.R.C., by American Cancer Society grant IM 457 and an American Heart Association Established Investigator award to E.L., and by an NIH Clinical Investigator Award to D.B.D.
26 July 1989; accepted 13 November 1989

Molecular Cloning of the Thyrotropin Receptor

MARC PARMENTIER, FREDERICK LIBERT, CARINE MAENHAUT, ANNE LEFORT, CATHERINE GÉRARD, JASON PERRET, JACQUELINE VAN SANDE, JACQUES E. DUMONT, GILBERT VASSART

The pituitary hormone thyrotropin, or thyroid-stimulating hormone (TSH), is the main physiological agent that regulates the thyroid gland. The thyrotropin receptor (TSHR) was cloned by selective amplification with the polymerase chain reaction of DNA segments presenting sequence similarity with genes for G protein–coupled receptors. Out of 11 new putative receptor clones obtained from genomic DNA, one had sequence characteristics different from all the others. Although this clone did not hybridize to thyroid transcripts, screening of a dog thyroid complementary DNA (cDNA) library at moderate stringency identified a cDNA encoding a 4.9-kilobase thyroid-specific transcript. The polypeptide encoded by this thyroid-specific transcript consisted of a 398–amino acid residue amino-terminal segment, constituting a putative extracellular domain, connected to a 346-residue carboxyl-terminal domain that contained seven putative transmembrane segments. Expression of the cDNA conferred TSH responsiveness to *Xenopus* oocytes and Y1 cells and a TSH binding phenotype to COS cells. The TSHR and the receptor for luteinizing hormone–choriogonadotropin constitute a subfamily of G protein–coupled receptors with distinct sequence characteristics.

THYROTROPIN (TSH) STIMULATES the function and the proliferation of thyrocytes and induces the expression of differentiation (*1*). Most of its effects are mediated by adenosine 3′,5′-monophosphate (cAMP) (*1*). Like the other pituitary and placental glycoprotein hormones [follicle-stimulating hormone (FSH), luteinizing hormone (LH), and choriogonadotropin (CG)], TSH is a heterodimer. All these hormones have identical α subunits; the β subunits, although possessing sequence similarity, are specific for each of the hormones (*2*). The activated TSH, FSH, and LH-CG receptors stimulate adenylyl cyclase in their respective target cells through the G protein G_s (*3*). In man, the TSH receptor (TSHR) can be the target of autoimmune reactions that lead to hyper- or hypostimulation of the thyroid gland by autoantibodies in Grave's disease and in idiopathic myxoedema, respectively (*4*).

With the use of the polymerase chain reaction (PCR) (*5*), we have taken advantage of the sequence similarity displayed by all known G protein–coupled receptors to amplify and clone new members of this gene family (*6*). The method involves the use of degenerate oligonucleotide primers corresponding to conserved regions in transmembrane segments of the known receptors. Previously, when applied to cDNA from thyroid tissue with primers corresponding to transmembrane segments III and VI, the method did not result in the cloning of TSHR. However, it led to the cloning of four new members of the G protein–coupled receptor family (*6*).

As most G protein–coupled receptor genes do not contain introns in their coding sequence, we have used a similar strategy with new sets of degenerate primers and

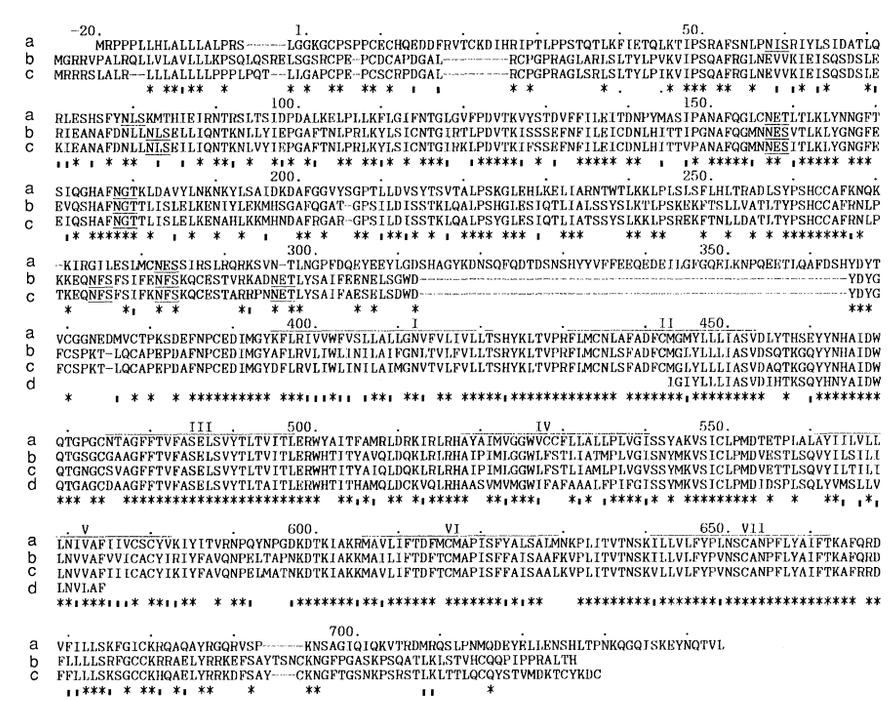

Fig. 1. Primary structure of the dog TSH receptor (a), as deduced from the nucleic acid sequence of the clone dTSHR (*18*). The sequence was aligned (*19*) with full-length rat (b) and pig (c) LH-CG receptor sequences (*16, 17*) and with the HGMP09 partial sequence (d). Numbering is given from the predicted first residue of the mature polypeptide (*12*). Every tenth residue is marked with a dot. Identical residues and conservative replacements in TSHR and the LH-CG receptors are indicated by an asterisk and a vertical bar, respectively. Potential sites for N-linked glycosylation are underlined. Putative transmembrane segments are overlined and numbered I through VII. Lambda phages containing dTSHR inserts were subcloned in M13 and sequenced on both strands (Applied Biosystems, Model 370A sequencer) by a combination of forced cloning and exonuclease III deletions (*20*).