usual strategy for the self-propagation of selfish DNA that can also function as a postzygotic isolating mechanism to facilitate an ongoing process of speciation.

REFERENCES AND NOTES

1. L. E. Orgel and F. H. C. Crick, *Nature* **284**, 604 (1980); W. F. Doolittle and C. Sapienza, *ibid.*, p. 601.
2. U. Nur, J. H. Werren, D. G. Eickbush, W. D. Burke, T. H. Eickbush, *Science* **240**, 512 (1988).
3. M. G. Kidwell, J. F. Kidwell, J. A. Sved, *Genetics* **86**, 813 (1977); G. Picard, *Biol. Cell.* **31**, 235 (1978).
4. The SP strain was derived from beetles collected in 1989 in a rice warehouse in Senoko, Singapore; the US strain was collected in a farmer's corn bin in Georgia in 1980.
5. R. W. Beeman, K. S. Friesen, R. E. Denell, unpublished data.
6. R. W. Beeman and J. J. Stuart, *J. Econ. Entomol.* **83**, 1745 (1990).
7. R. W. Beeman, T. R. Johnson, S. M. Nanis, *J. Hered.* **77**, 451 (1986).
8. H. A. Orr, *Evolution* **43**, 180 (1989); *Genetics* **116**, 555 (1987).
9. P. Hutter, J. Roote, M. Ashburner, *Genetics* **124**, 909 (1990).
10. P. Christie and M. R. Macnair, *J. Hered.* **75**, 510 (1984); *Evolution* **41**, 571 (1987).
11. M. J. Wade, personal communication.

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A Conformation of Cyclosporin A in Aqueous Environment Revealed by the X-ray Structure of a Cyclosporin-Fab Complex

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The conformation of the immunosuppressive drug cyclosporin A (CsA) in a complex with a Fab molecule has been established by crystallographic analysis to 2.65 angstrom resolution. This conformation of CsA is similar to that recently observed in the complex with the rotamase cyclophilin, its binding protein in vivo, and totally different from its conformation in an isolated form as determined from x-ray and nuclear magnetic resonance analysis. Because the surfaces of CsA interacting with cyclophilin or with the Fab are not identical, these results suggest that the conformation of CsA observed in the bound form preexists in aqueous solution and is not produced by interaction with the proteins.

Cyclosporin A (CsA), an immunosuppressive drug that acts as an inhibitor of T cell activation (*1*), forms a complex in vivo with cyclophilin (CYP) (*2*), a cis-trans isomerase, and can inhibit its activity in vitro (*3*, *4*). The molecular mechanisms of immunosuppressive activity are not understood. Furthermore, the relation between immunosuppressive activity and inhibition of rotamase activity has recently been questioned [for review, see (*5*, *6*)]. Knowledge of the conformation of the drug that is recognized by CYP is central to an understanding of its mechanism of action. Two crystallographic (*7*, *8*) and two nuclear magnetic resonance (NMR) analyses in different apolar solvents (*9*) of isolated CsA yielded superposable structures, suggesting that CsA is a rigid molecule with a distinctive main-chain conformation. The conformation of CsA in aqueous solution has never been studied because of its poor solubility. Two NMR studies of CsA in a complex with CYP yielded, however, a conformation of CsA with a totally rearranged backbone conformation (*10*, *11*). The cis peptide bond found between residues MeLeu^9 and MeLeu^{10} (Me, *N*-methyl) in free CsA was replaced by a trans peptide bond in the conformation of CsA in interaction with CYP.

Is the radical conformational rearrangement of the whole CsA molecule a result of its binding to CYP (*12*, *13*)? Although some conformational adaptation may occur upon interaction of CsA with an antibody combining site, such drastic changes are not expected from its free conformation in solution. We have established by crystallographic analysis to 2.65 Å resolution the structure of CsA in a complex with the Fab of an antibody and analyzed its interaction with the antibody combining site.

The monoclonal antibody to CsA R454511, which was chosen for crystallographic analysis of an Fab-CsA complex, is an immunoglobulin G1/κ, known from immunochemical data to strongly recognize residues 2 through 5 and 11 and weakly recognize residues 1 and 9 of CsA (*14*). Messenger RNA sequencing of its variable region revealed an unusually long H3 loop (*15*). The structure of CsA in the Fab R454511–CsA complex, derived here from our crystallographic analysis, is shown in Fig. 1A. The difference electron density map clearly shows the shape of the CsA cycle as well as the location of side chains. The conformation is different from that of the isolated form (Fig. 1B), also obtained by x-ray diffraction (*8*). However, the conformation of CsA in interaction with the antibody closely resembles that recently observed, with NMR analyses, in a complex with CYP (*10*, *11*). The conformation of isolated CsA (Fig. 1B) contains two antiparallel β strands formed by residues 11 to 3 and 4 to 7. All four nonmethylated main-chain nitrogens are involved in internal hydrogen bonds with carbonyl groups. In the structure of CsA shown in Fig. 1A, no internal hydrogen bonds are formed. Instead, main-chain nitrogens and carbonyls are available for hydrogen bonding to the protein or to the solvent. The internal structure of the cycle is mainly hydrophobic because most main-chain *N*-methyls, as well as the side chain of MeVal^5, point to the inside of the cycle.

The connection between residues MeLeu^9 and MeLeu^{10} is best fit by a trans peptide bond, as in the conformation of CsA in interaction with CYP (*10*, *11*), in contrast to the cis peptide bond found in isolated CsA (Fig. 1B). Although topologically similar overall, the conformations of CsA bound to the Fab or to CYP (*10*, *11*) are not identical. For example, when CsA is bound to the Fab, MeBmt^1 does not fold back onto the molecule as when CsA is bound to CYP (MeBmt, 3-hydroxy-4-methyl-2-methylamino-6-octenoic acid). Differences, however, are small compared to those of the totally unrelated conformations of CsA in complex with the Fab (Fig. 1A) and isolated CsA (Fig. 1B).

The antibody is unlikely to distort CsA through a rotamase activity that would mimic CYP. Indeed, the binding modes of the Fab and of CYP are different: CYP is known from NMR experiments to recognize residues 1 through 3 and 9 through 11 of CsA (*10*, *11*). Crystallographic analysis of the Fab-CsA complex clearly shows that residues 9 and 10 and the side chain of residue 1 are not involved, whereas residues 11 and 2 to 5 and the main chain of residue 1 are critical for binding (Fig. 2). These latter residues are also those found from immunochemical data to be important for recognition (*14*).

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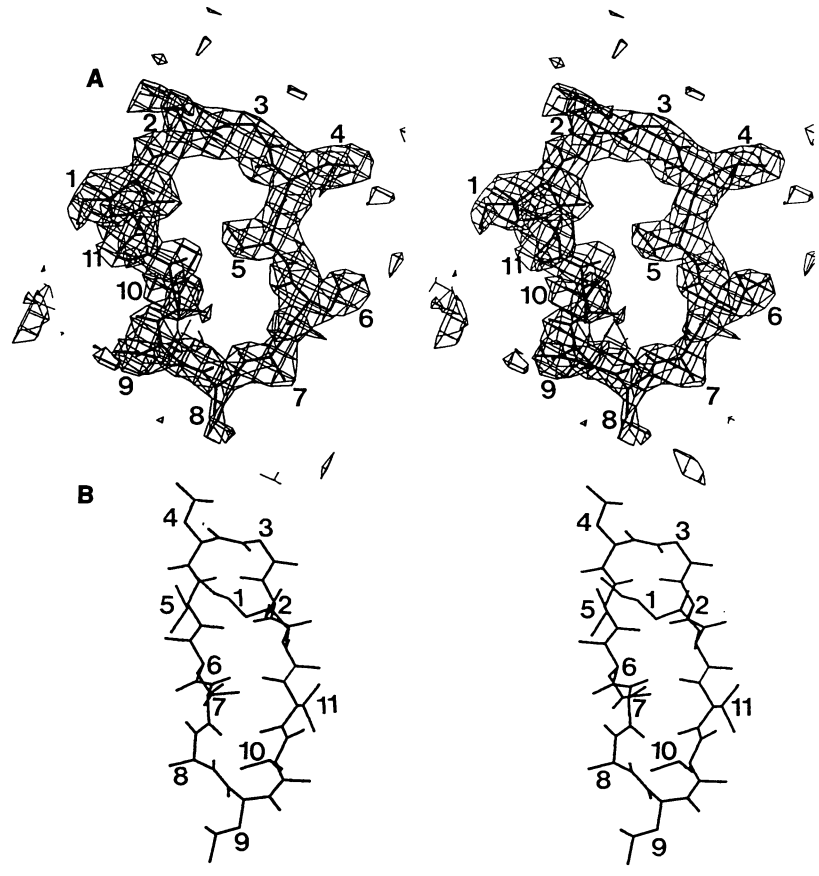

Fig. 1. X-ray structures of (**A**) CsA in the complex with the Fab fragment R454511 (this study) and (**B**) isolated CsA (*8*). Orientations were chosen so that the side chain of residue 1 points up and that of residue 11 points down relative to the plane defined by the cycles, emphasizing the lack of conformational similarity between the two structures. The CsA part of the complex was omitted from the model used to calculate the difference electron density shown in (A) ($F_{obs} - F_{calc}$ synthesis, contoured at 1.4σ). Monoclonal antibody R454511 to CsA was prepared according to Quesniaux *et al.* (*14*). Crystals belong to the orthorhombic space group $P2_12_12_1$, with a = 52.6 Å, b = 70.2 Å, and c = 118.4 Å with one molecule of complex per asymmetric unit (*28*). Native data sets were collected to 2.6 Å resolution (on a Xentronics area detector at the European Molecular Biology Laboratory, Heidelberg, Germany; at the University of California at San Diego with N. Xuong's data collection facilities; and on a Siemens area detector). The merging factor R_{sym} to 2.6 Å resolution is 0.087, including all measurements (63,899 reflections) for 14,219 independent reflections with 220 intensities less than or equal to zero. The structure was solved by molecular replacement with a composite structure, formed by the light chain of Fab J539 (*29*) and the heavy chain of Fab KOL (*30*), as a known model structure (*31*). This molecular replacement solution was first improved by rigid-body refinement of the four basic Fab domains [CORELS (*32*)]. The refinement of the whole structure was conducted with a procedure alternating energy minimizations [PROLSQ (*33*) and X-PLOR (*34*)] and dynamic refinements [GROMOS (*35*)], followed by model building [FRODO (*36*)]. A polyalanine chain was built gradually into the density attributed to CsA through the use of $3F_{obs} - 2F_{calc}$ difference maps, until the sequence MeBmtAbuSar (Abu, L-α-aminobutyric acid) could be clearly identified and the CsA side chains (MeBmtAbuSarMeLeuValMeLeuAla-D-AlaMeLeuMeLeuMeVal) built into density. The mean value of the B factors is 16.3 Å^2 for CsA residues as compared to 9.9 Å^2 for Fab residues. The current crystallographic R factor is 0.185 for 11,236 reflections between 8 and 2.65 Å with a $\sigma(I)/I$ greater than 2. The refined coordinates will be deposited in the Brookhaven Protein Data Bank.

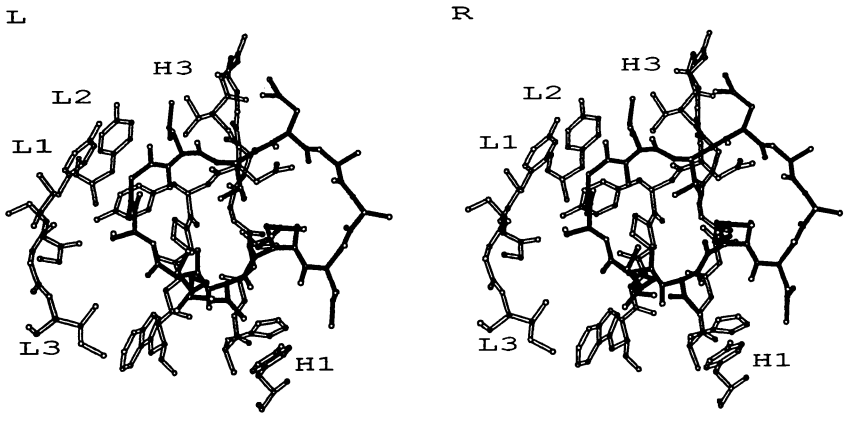

Fig. 2. Stereo view of the interaction between CsA and the combining site of the monoclonal Fab fragment R454511. Cyclosporin A is shown in black lines and the antibody residues interacting with CsA in white lines. Antibody residues within 4.0 Å of CsA residues were selected in the drawing.

The conformation of isolated CsA might arise from the conditions used in crystallographic studies and NMR studies in aprotic solvents, which do not favor the formation of hydrogen bonds with the environment. Aprotic solvents are known to influence peptide conformation. For example, circular dichroism studies have shown that trifluoroethanol increases the helical population of peptides (*16*, *17*). We observed here a structure with a higher hydrogen-bonding potential than that in the isolated form. The striking structural similarity between CsA in complex with CYP and with the Fab suggests that this conformation of CsA exists in hydrophilic environments. The binding of CsA to CYP would then require only minor conformational adjustments, if any, and not the radical transformation from the structure shown in Fig. 1B to that in Fig. 1A.

The binding protein for FK506 (FKBP), another immunosuppressive drug, is also a rotamase (*18*, *19*) but structurally unrelated to cyclophilin (*20*). Here also a cis amide bond of the isolated FK506 is trans in the complex with FKBP (*21*). However, NMR analysis of FK506 in $CDCl_3$ reveals the existence of both cis and trans rotamers (*22*), so preferential binding of the trans rotamer present in solution cannot be excluded (*23*). Our observations support the hypothesis that immunosuppressive drugs are not substrates of their respective binding proteins (*24*) but solely inhibitors of the rotamase activity.

A mechanism for the isomerization of peptide substrates has been proposed on the basis of the crystallographic structures of a tetrapeptide-CYP (*20*) complex and FK506-FKBP (*21*) complex, and the CsA binding site on CYP has been shown to be coincident with the substrate binding site (*20*). However, it has not been established so far that the isomerases induce a conformational change of the immunosuppressants. Knowledge of a solution structure of CsA is of particular significance for understanding its mechanism of action and designing more potent immunosuppressive drugs.

REFERENCES AND NOTES

1. J. F. Borel, *Immunology* **31**, 631 (1976).
2. R. E. Handschumacher, M. W. Harding, J. Rice, R. J. Drugge, D. W. Speicher, *Science* **226**, 544 (1984).
3. N. Takahashi, T. Hayano, M. Suzuki, *Nature* **337**, 473 (1989).
4. G. Fischer, B. Wittmann-Liebold, K. Lang, T. Kiefhaber, F. X. Schmid, *ibid.*, p. 476.
5. S. L. Schreiber, *Science* **251**, 283 (1991).
6. F. McKeon, *Cell* **66**, 823 (1991).
7. T. J. Petcher, H. P. Weber, A. Rüegger, *Helv. Chim. Acta* **59**, 1480 (1976).
8. H.-R. Loosli *et al.*, *ibid.* **68**, 682 (1985).
9. H. Kessler, H.-R. Loosli, H. Oschkinat, *ibid.*, p. 661.
10. C. Weber *et al.*, *Biochemistry* **30**, 6563 (1991).
11. S. W. Fesik *et al.*, *ibid.*, p. 6574.
12. K. Wüthrich *et al.*, *Science* **254**, 953 (1991).
13. W. L. Jorgensen, *ibid.*, p. 954.
14. V. F. J. Quesniaux, R. Tees, M. H. Schreier, R. M. Wenger, M. H. V. Van Regenmortel, *Mol. Immunol.* **24**, 1159 (1987).
15. D. Schmitter *et al.*, *ibid.* **27**, 1029 (1990).
16. H. J. Dyson, M. Rance, R. A. Houghten, P. E. Wright, R. A. Lerner, *J. Mol. Biol.* **201**, 201 (1988).
17. B. Prêcheur, O. Siffert, O. Bârzu, C. T. Craescu, *Eur. J. Biochem.* **201**, 67 (1991).
18. J. J. Siekierka, S. H. Y. Hung, M. Poe, C. S. Lin, N. H. Sigal, *Nature* **341**, 755 (1989).
19. M. W. Harding, A. Galat, D. E. Uehling, S. L. Schreiber, *ibid.*, p. 758.
20. J. Kallen *et al.*, *ibid.* **353**, 276 (1991).
21. G. D. Van Duyne, R. F. Standaert, P. A. Karplus, S. L. Schreiber, J. Clardy, *Science* **252**, 839 (1991).
22. M. K. Rosen, R. F. Standaert, A. Galat, M. Nakatsuka, S. L. Schreiber, *ibid.* **248**, 863 (1990).
23. R. L. Stein, *Curr. Biol.* **1**, 234 (1991).
24. T. Hultsch, M. W. Albers, S. L. Schreiber, R. J. Hohman, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 6229 (1991).
25. R. A. Crowther, in *The Molecular Replacement Method*, M. G. Rossmann, Ed. (Gordon and Breach, New York, 1972), pp. 173–178.
26. M. Cygler and W. F. Anderson, *Acta Crystallogr.* **A44**, 38 (1988).
27. ———, *ibid.*, p. 300.
28. D. Altschuh *et al.*, *J. Mol. Biol.* **209**, 177 (1989).
29. S. W. Suh *et al.*, *Proteins* **1**, 74 (1986).
30. M. Marquart, J. Deisenhofer, R. Huber, W. Palm, *J. Mol. Biol.* **141**, 369 (1980).
31. D. Altschuh *et al.*, unpublished results. The first two peaks given by the fast rotation function (*25*) correspond to the orientations of the variable and constant domains, respectively, with search conditions following Cygler and Anderson (*26*). Translation vectors can be unambiguously defined with the program TSFGEN (I. Tickle, CCP4 package) for both constant and variable domains when data between 20 and 4 Å resolution are included (*27*).
32. J. L. Sussman, S. R. Holbroock, G. M. Church, S. H. Kim, *Acta Crystallogr.* **A33**, 800 (1977).
33. W. A. Hendrickson and J. H. Konnert, in *Computing in Crystallography*, R. Diamond, S. Rameseshan, K. Venkatesan, Eds. (Indian Academy of Sciences, Bangalore, 1980), p. 13.01.
34. A. T. Brünger, *X-PLOR Manual, Version 2.1* (Yale University, New Haven, CT, 1990).
35. M. Fujinaga, P. Gros, W. F. Van Gunsteren, *J. Appl. Crystallogr.* **22**, 1 (1989).
36. A. Jones, in *Frodo: A Graphics Fitting Program for Macromolecules*, D. Sayre, Ed. (Oxford Univ. Press, Oxford, 1982), pp. 303–317.

37. We are grateful to N. Xuong for allowing us to use his data collection facilities (University of California at San Diego), P. Tücker for help with data collection at the European Molecular Biology Laboratory, and A. Mitschler for his excellent assistance during data collection. We thank D. Moras and M. H. V. Van Regenmortel for constant interest and critical comments on the manuscript.

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Competition for Overlapping Sites in the Regulatory Region of the *Drosophila* Gene *Krüppel*

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A 730–base pair element regulates expression of the *Drosophila* gap gene *Krüppel* (*Kr*) in response to the fly anterior morphogen *bicoid* (*bcd*). Two hormone receptor–like proteins, encoded by the genes *knirps* (*kni*) and *tailless* (*tll*), bind specifically to the element. In vitro, *kni* protein competes with the homeodomain-containing *bcd* protein in binding to a 16–base pair target sequence. In vivo experiments suggest that both *kni* and *tll* act as competitive repressors of *bcd*-mediated activation of *Kr*. These results suggest a mechanism by which developmental genes can be regulated in response to an activating morphogen gradient antagonized by repressors.

In vertebrates, hormone receptors are transcription factors involved in developmental processes, cell differentiation, and morphogenetic events (*1*). They respond to extracellular ligands, including steroid hormones, vitamin D, or retinoic acid (*1*). In addition to having the common structure of the DNA-binding domain characterized by a pair of Cys-containing zinc fingers (*2*), these transcription factors share a varying degree of sequence similarity in the COOH-terminal ligand-binding domain (*1*). On the basis of sequence similarity, eight *Drosophila* genes have been grouped as putative members of this superfamily of nuclear hormone receptors (*3*). Two of them, *tll* (*4*) and *kni* (*5*, *6*), are gap genes, which are the first genes in the segmentation gene hierarchy to be expressed in *Drosophila* embryonic development (*7*). Consistent with their mutant phenotypes, *kni* and *tll* are expressed in spatially restricted domains along the anterior-posterior axis of the blastoderm embryo, specifying abdominal segments (*4*) and terminal pattern elements (*6*), respectively. Genetic analysis suggests that both genes act as negative regulators of a third gap gene, *Kr* (*8*, *9*), which is required for the formation of thorax and anterior abdominal segments (*7*, *10*). We show that *kni* and *tll* proteins interact with multiple specific sites within the 730-bp regulatory region (*Kr* 730) of the *Kr* gene (*11*). This previously characterized *Kr* 730 element is controlled by the morphogenetic *bcd* protein (BCD) gradient (*12*) and is sufficient to direct correctly localized activation of a reporter gene in the central domain of *Kr* gene expression in early blastoderm embryos (*11*).

Using bacterially expressed *kni* protein (KNI) and the DNA binding region of *tll* protein [TLL(181-517)], we found, with in vitro footprinting (*13*), a single strong KNI binding site and seven TLL(181-517) binding sites on the *Kr* 730 element (Figs. 1 and 2). Palindromic or tandemly repeated sequences, such as those observed with different vertebrate hormone receptors (*14*), were not observed in the KNI and TLL(181-517) consensus sequences (*15*).

The binding sites of TLL and the KNI binding site overlap with previously detected binding sites of the homeodomain *bcd* protein (BCD) (*11*); KNI binds to one region and TLL binds to all regions protected by BCD (Figs. 1 and 2). We analyzed the single BCD site overlapped by a KNI binding site, a 16-bp sequence: 5′-ACTGAACTAAATCCGG-3′. KNI and BCD competed for binding to the 16-bp sequence in vitro. Increasing amounts of BCD competed for the binding of KNI. Conversely, when increasing amounts of KNI were added to a constant amount of BCD, BCD was replaced by KNI binding (*15*). These experiments demonstrate that each of the two proteins can bind but that their binding is mutually exclusive.

To examine a possible functional interaction of KNI and BCD with the 16-bp element in vivo, we transfected tissue culture cells (*16*) with reporter gene constructs containing the bacterial chloramphenicol acetyltransferase (CAT) gene under the control of the 16-bp element (*17*). No effect on the basal level of CAT gene expression was observed when plasmid DNA that contained *kni* was co-transfected. In contrast, plasmid DNA that contained *bcd* caused a dosage-dependent increase in CAT expression (Fig. 3A), indicating that the 16-bp element mediated BCD-dependent activation of gene expression. When KNI was introduced along with BCD (Fig. 3B), BCD-dependent CAT gene activation was suppressed in a manner responsive to the dosage of KNI. KNI did not make the reporter gene inactivable (Fig. 3C), but instead increased the amount of BCD necessary for activation. No repression was observed when the DNA-binding domain of KNI was mutated such that the first Cys in the second zinc finger was replaced by a Leu residue.

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