'10. 7. 28. (Wed.)
Shogo HASHIZUME (M1)

Chemistry and Biology of FK506

— S. L. Schreiber's pioneering work of the "chemical biology"

Stuart L. Screiber, Ph.D. was born in 1956. He received B.A. degree at the University of Virginia in 1977, and Ph.D. at Harvard University under the supervision of R. B. Woodward and Y. Kishi in 1981. After that, he joined the faculty at Yale University and was promoted to Associate Professor in 1984 and Full Professor in 1986. In 1988, he returned to Harvard. Now, he is Professor of Chemistry and Chemical Biology at Harvard University and Director of Chemical Biology of the Broad Institute of Harvard and MIT.

He is well-known for his strategy that synthesized molecules can be utilized as probe of biological molecule and suggested one field of science, "chemical biology". As the pioneer in this field, his first famous work is **synthesis of FK506 and biological study using synthesized FK506 and its analogues**.

→ ***Today's theme***

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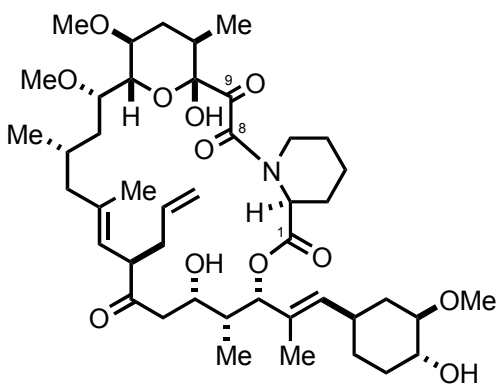
1. Introduction and Background

1-1. FK506 and other immunosupressants

J. Am. Chem. Soc. **1987**, *109*, 5031
J. Antibiotics **1987**, *40*, 1249
Immun. Today **1989**, *10*, 6



FK506 (Tacrolimus)

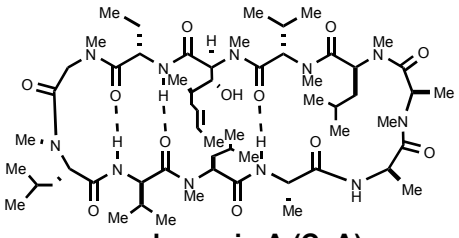
— FK506 was isolated from *Streptomyces tsukubaensis* by research group of Fujisawa Pharma. Co. in 1987.

— FK506 shows more potent immunosurpressive activity than cyclosporins, both *in vivo* and *in vitro*.

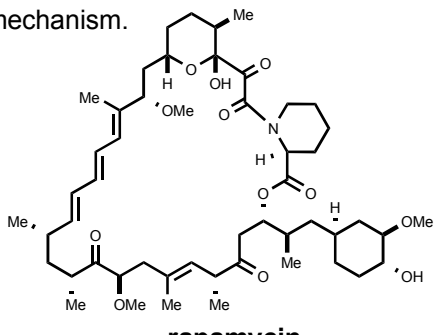
— Tacrolimus has been used for immunosupressant after organ transplant, myasthenia gravis, rheumatism, etc. since authorized in 1993.

— Today, Astellas Pharma Inc. is selling Tacrolimus as Prograf® and Graceptor®.

— CsA and rapamycin are similar immunosupressants in view of acting mechanism. They inhibit signal transduction pathway in T lymphocyte selectively.



cyclosporin A (CsA)



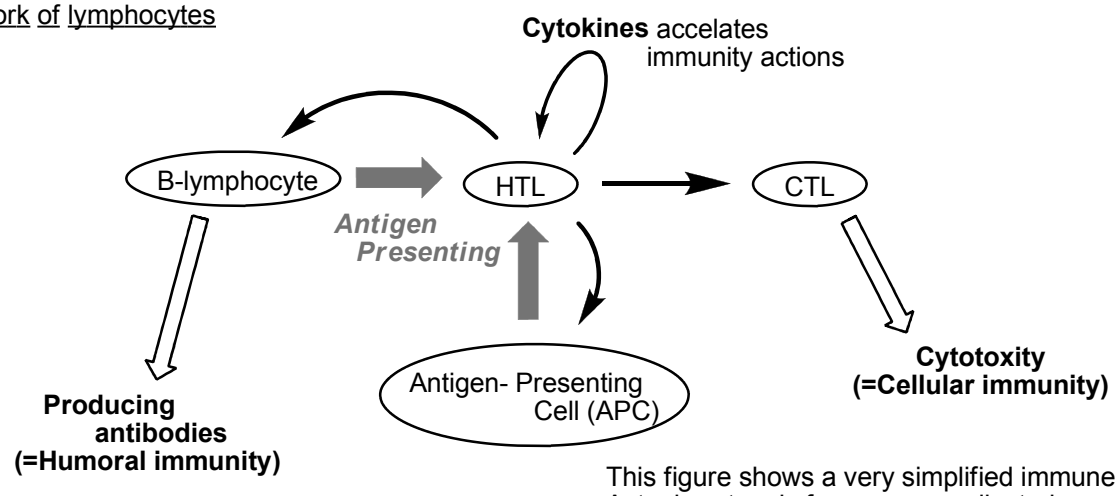
rapamycin

1-2. Immune system as background of this work

ref) 菊池浩吉,菊池由里『最新免疫学図説』メディカルカルチュア 1995
D.Male著,多田富雄訳『免疫学イラストレイテッド(原著第3版)』南江堂 1995

The immune system
- Innate immunity
- Adaptive immunity
  - Humoral immunity : served by B-lymphocyte
  - Cellular immunity : served by T-lymphocyte
    - Helper T-lymphocyte (HTL)
    - Cytotoxic T-lymphocyte (CTL)

Network of lymphocytes



This figure shows a very simplified immune system.
Actual system is far more complicated.

Interleukin-2 (IL-2) : one of the cytokines

Secreted by helper T-lymphocyte when HTL was stimulated by antigen via T-cell receptor (TCR)
Activates grouwth and differentiation of HTL, CTL, etc.

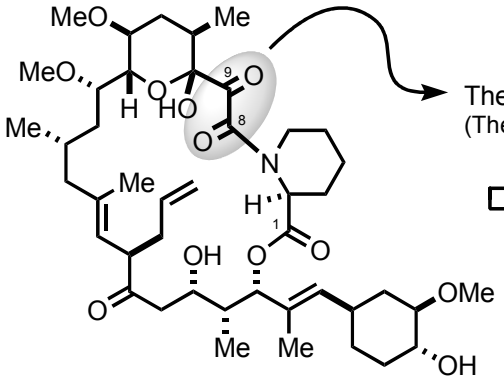
It was already clear when Schreiber set out this work that FK506's biological activity has relationship with IL-2.
M. J. Tocci *et al*. had showed that FK506 apperantly inhibits the accumulation of IL-2 gene mRNA.

M. J. Tocci *et al.*
J. Immunology **1989**, *143*, 718

But FK506's inhibition mechanism at molecular level was still unclear...
Schreiber's work made it clear *starting from total synthesis* of FK506.

2. Total Synthesis of FK506 and ^{13}C-labelled FK506

S. L. Schreiber *et al.*
J. Org. Chem. **1989**, *54*, 9
J. Org. Chem. **1989**, *54*, 15
J. Org. Chem. **1989**, *54*, 17
J. Org. Chem. **1989**, *54*, 4267
J. Am. Chem. Soc. **1990**, *112*, 5583



FK506

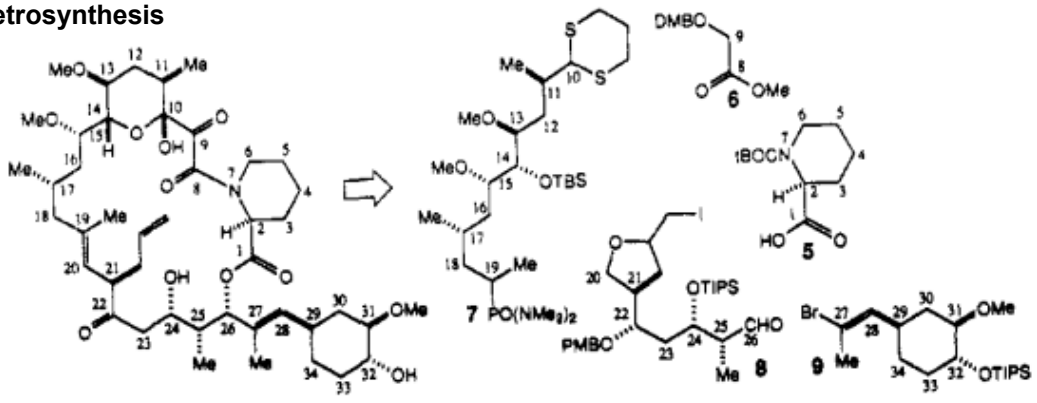
These two carbonyl are involved in binding to the target molecule.
(The reason will be stated later.)

Labelling these carbon (C_8 and C_9) would reveal the details of binding mechanism ??

The key is a route enabling to **introduce this C-2 unit independently.**

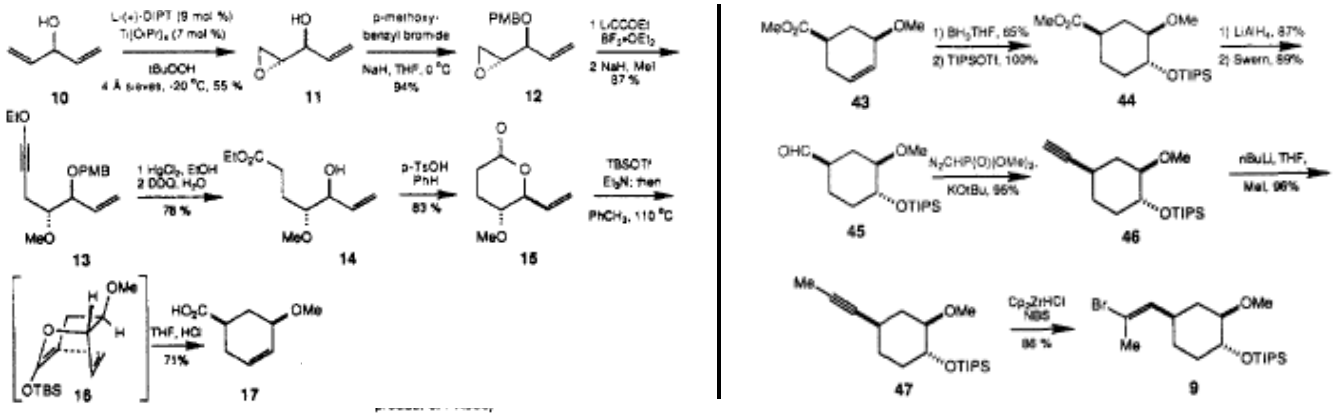
considering this strategy....

Retrosynthesis

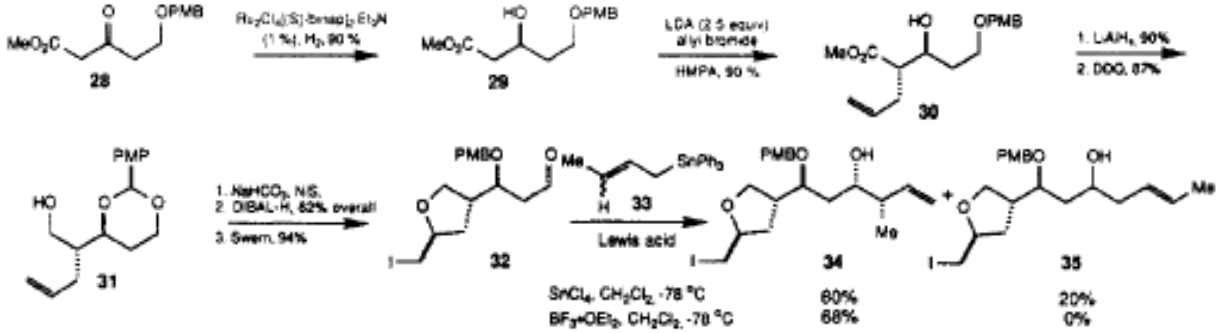


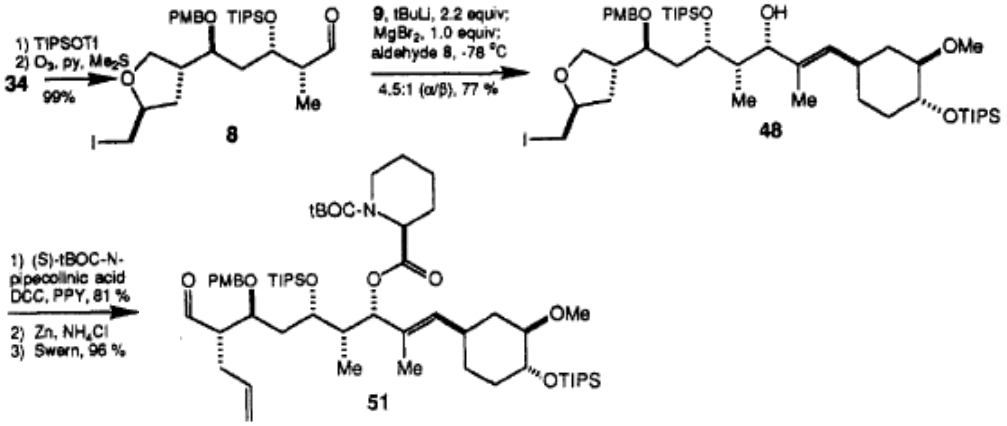
FK-506 1

Synthesis of C_{27}-C_{34} unit

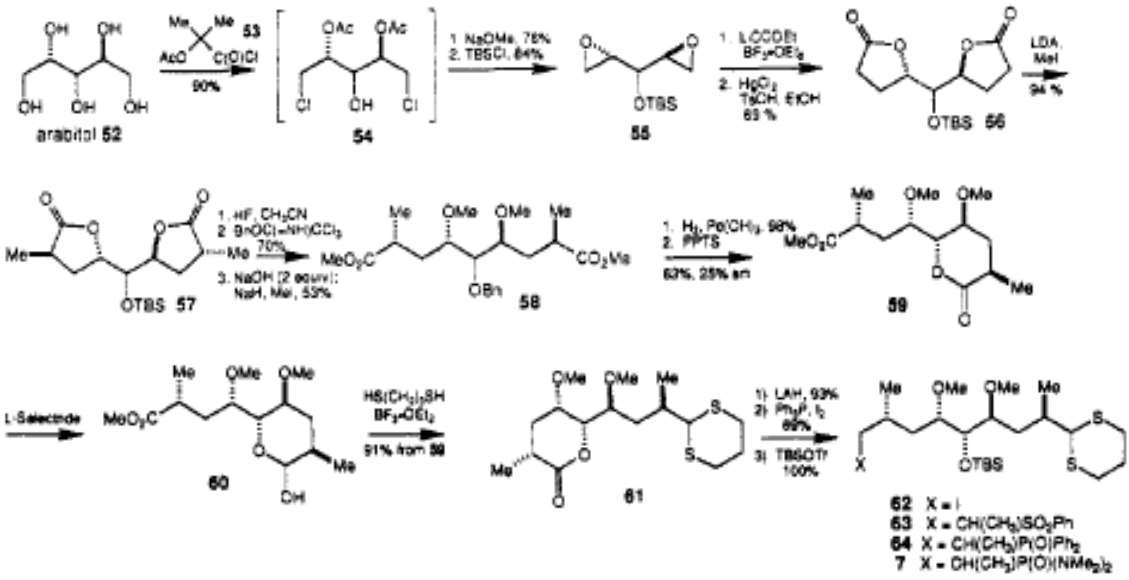


Synthesis of C_{20}-C_{26} unit followed by coupling with C_{27}-C_{34} unit and C_1-N_7 unit

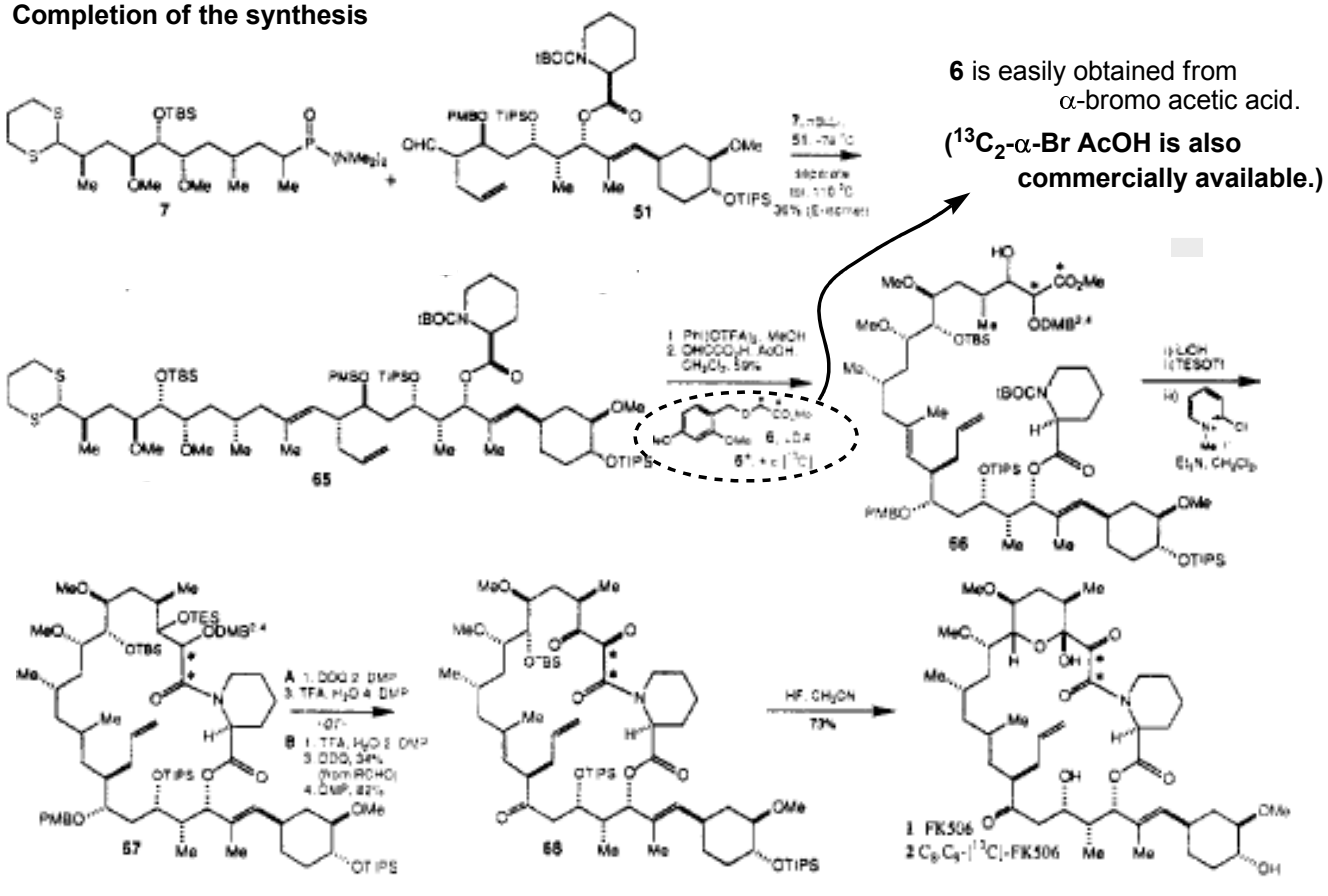




Synthesis of C_{10}-C_{19} unit



Completion of the synthesis



6 is easily obtained from α-bromo acetic acid.

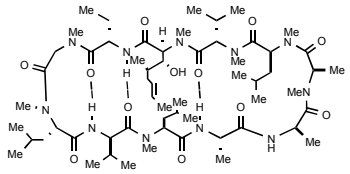
($^{13}C_2$-α-Br AcOH is also commercially available.)

3. Exploring of Molecular Target of FK506 and Its Binding Mechanism

3-1. Identification of FK506-binding protein

In case of related immunosupressant cyclosporin A (CsA)...

G. Fischer *et al. Nature* **1989**, *337*, 476
N. Takahashi *et al. Nature* **1989**, *337*, 473



cyclosporin A (CsA)

binding → **cyclophilin**

target protein of CsA and
*cis-trans p*eptidyl-prolyl isomerase

FK506

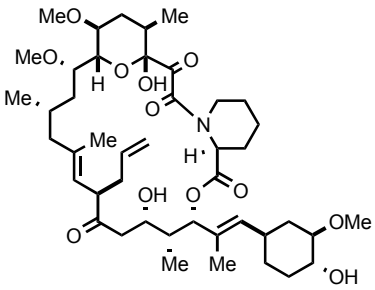
also binding ???

At first, FK506's target seems to be also cyclopilin because the two agents showed similar bioactivity.

But these have little structual similarity..

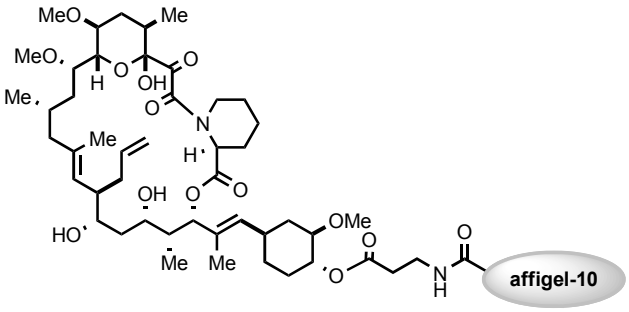
Schreiber and co-workers tried to **identify the FK506-binding protein (FKBP).**

S. L. Schreiber *et al. Nature* **1989**, *341*, 758



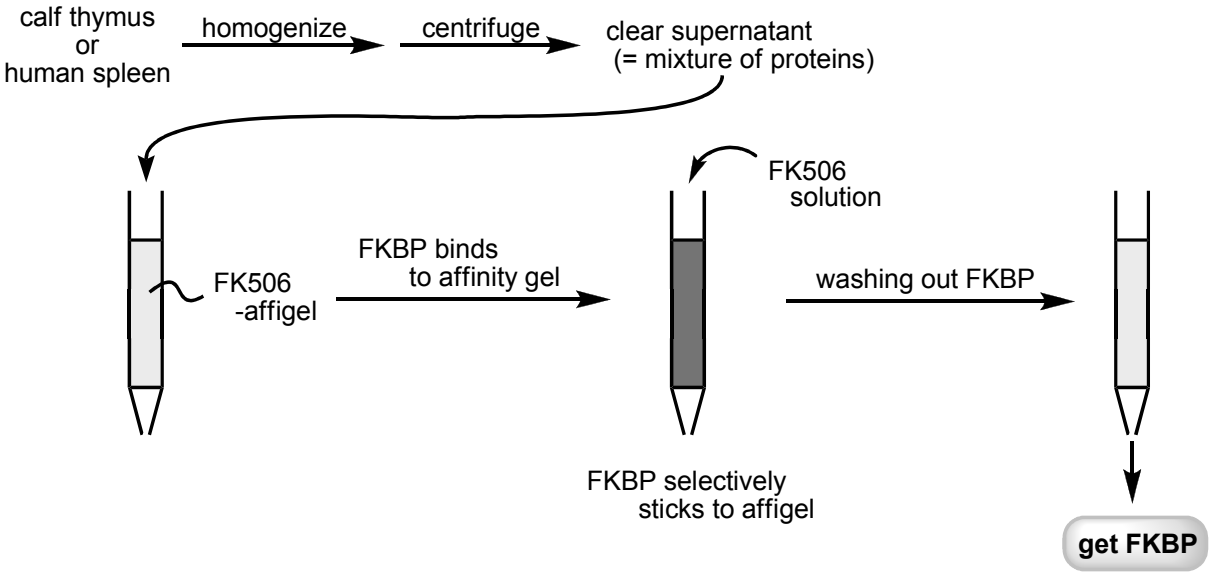
FK506

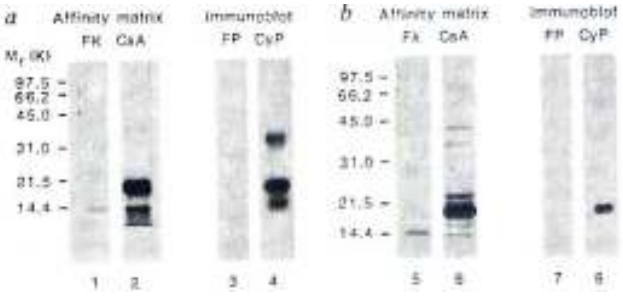
1. L-Selectride
2. $N_3(CH_2)_2COCl$
3. $HS(CH_2)_3SH$
4. affigel-10



affigel-10

FK506-affigel

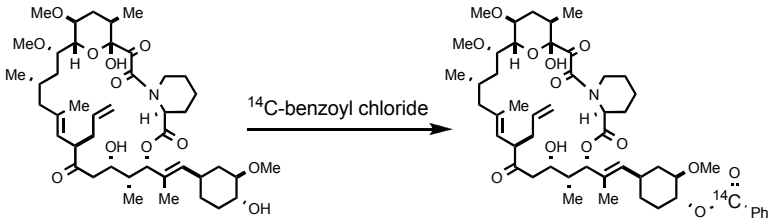




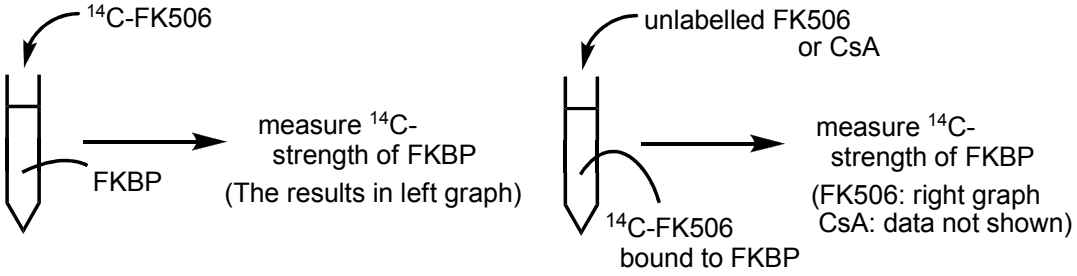
They succeeded in isolating FKBP by this method. (lane 1 and 5 in figure)
And this isolated FKBP was anti-cyclophiline IgE. The result showed cyclophilin and FKBP are antigenically different. (lane 3 and 7)

a: from bovine thymus
b: from human spleen
immunoblot: react with anti-cyclophilin IgE

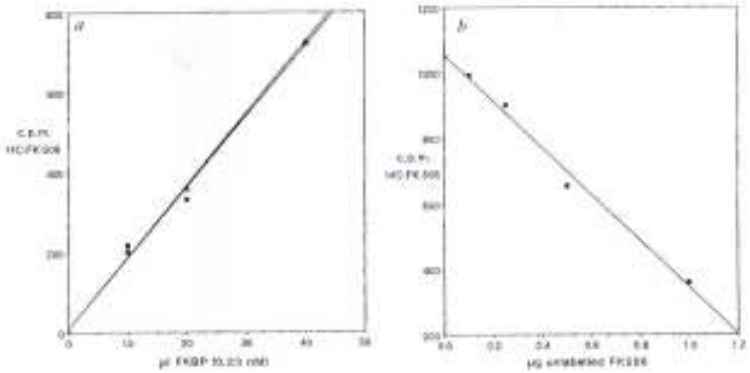
They tested this speculation by another experiment.
FK506 was ^{14}C-labelled and exposed assey with FKBP.

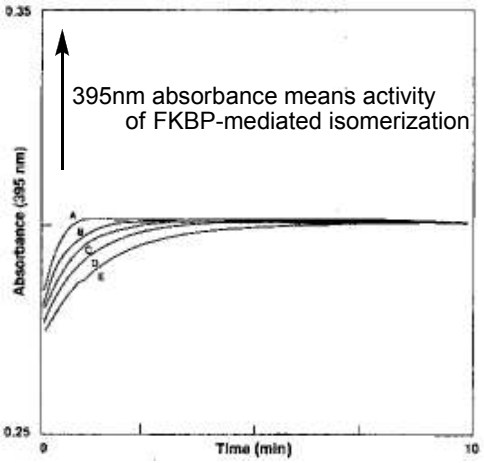


FK506 **32-[1-^{14}C]-benzoyl-FK506**



Unlabelled FK506 displaced ^{14}C-labelled FK506, but contrarily, CsA didn't.





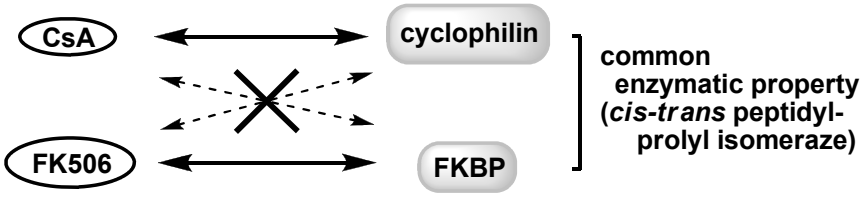
Isomerization activity of FKBP was also tested (right figure).
Suc-Ala-Ala-Pro-Phe-4-nitroanilide as model peptide-substrate.
(This experiment was developed by Fischer *et al*. (*Nature* **1989**, *337*, 476))

cis-trans isomerization activity was increased with FKBP (A), compared with exp. without FKBP (E).
And this FKBP activity was inhibited by FK506 relatively to the conc..
Furthermore, CsA had no effect about FKBP inhibition. (data not shown)

A: FKBP
B: FKBP with 27nM of FK506
C: FKBP with 54nM of FK506
D: FKBP with 270nM of FK506
E: control

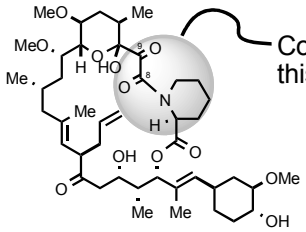
In addition to these experiments, FKBP's amino acid sequence didn't match cyclophilin's one.
And FKBP's sequence matched none of the sequence from gene database.
(= This isolated FKBP was **unknown and new enzyme**.)

This series of experiments showed...



3-2. Mechanism of FK506-FKBP binding

S. L. Schreiber *et al.*
Science **1990**, *248*, 863



FK506

Considering FKBP is *cis-trans* peptidyl-prolyl isomeraze, this group may be involved in the binding.

Two hypothetical mechanism of rotamease

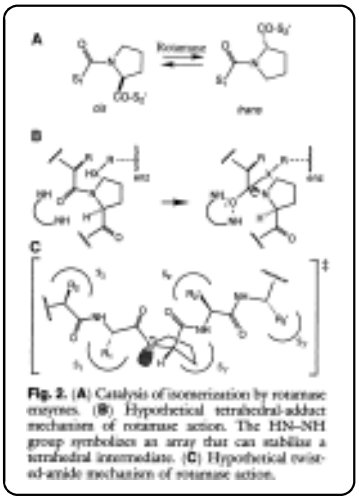


Fig. 2. (**A**) Catalysis of isomerization by rotamase enzymes. (**B**) Hypothetical tetrahedral-adduct mechanism of rotamase action. The HN–NH group symbolizes an array that can stabilize a tetrahedral intermediate. (**C**) Hypothetical twisted-amide mechanism of rotamase action.

FK506 binds to FKBP in tetrahedral-adduct mechanism or twisted-amide mechanism ??

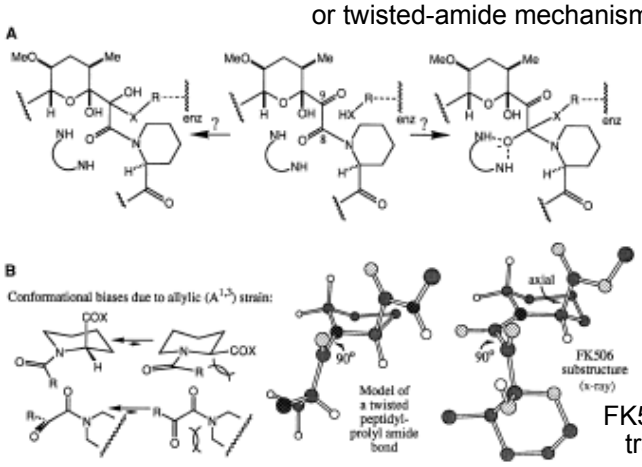


Fig. 3. Possible mechanisms of inhibition of FKBP by FK506 for (**A**) a tetrahedral adduct and (**B**) a twisted-amide surrogate.

FK506 binds as a mimic of transition state of peptide substrate??

→ **[8,9-^{13}C] FK506** was used to determine the binding mechanism.
(by ^{13}C-NMR measurement of labelled FK506 with or without recombinant-FKBP(rFKBP))

^{13}C-FK506 only

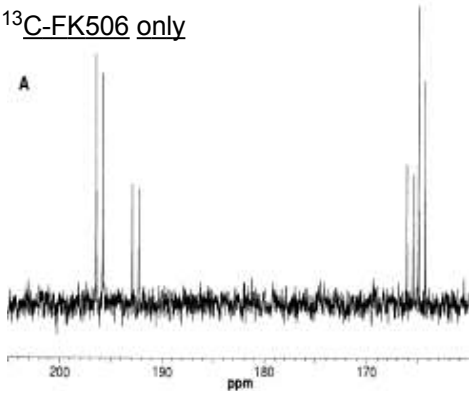


figure A: ^{13}C spectrum of [8,9-^{13}C]FK506

This two pairs coupled doublet peaks means *cis*- and *trans*-amide rotamers of FK506 in solution state.

^{13}C-FK506 with rFKBP

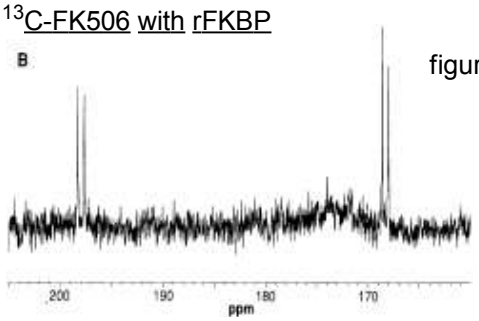
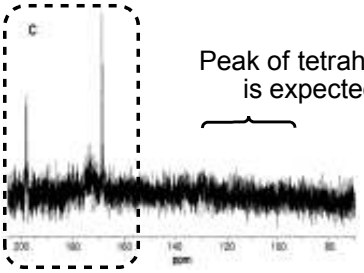


figure B: ^{13}C spectrum of complex between [8,9-^{13}C]FK506 and FKBP

FK506 takes a single rotamer in binding-state with FKBP.



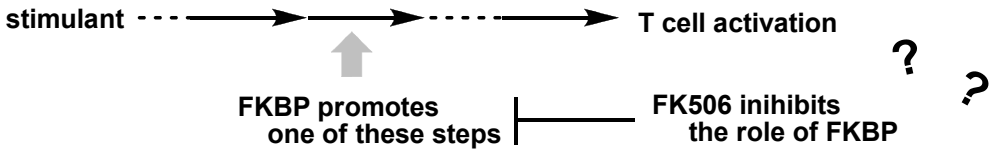
Peak of tetrahedral carbon is expected in this region...

This NMR study reveals that FK506 binds to FKBP not covalently but by **taking stabilized twisted-amide state** as mimic of the peptidyl-prolyl-*cis-trans* isomerization of its peptide-substrate.

4. Elucidation of Signal Transduction Pathway Inhibited by FK506

4-1. FKBP-mediated isomerization has a role for T-cell activation??

It was proved by the experiments above that FK506 binds to FKBP selectively.
And FKBP is identical to peptidyl-prolyl-*cis-trans* isomerase.
But does FKBP really have a crucial role for T cell activation pathway ???



This hypothetical signaling pathway involving FKBP is **doubtful**
because of several experimental results below.

Schreiber and co-workers conducted molecular cloning and overexpression of human-FKBP.
(It is for making recombinant FKBP for assay with some imunnosupressants and thier analogues.)

S. L. Schreiber *et al. Nature* **1990**, *346*, 671

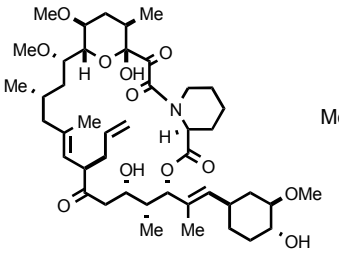
And Sigal *et al.* reported FK506 inhibits calcium-dependent lyphokine gene transcription.

N. H. Sigal *et al. J. Immun.* **1990**, *144*, 251

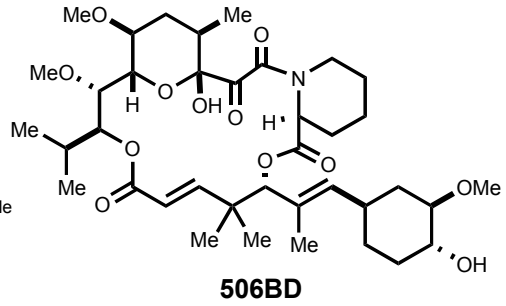
Judging from FKBP's amino acids sequence, however, FKBP is probably neither a direct activator of gene-transcription nor a calcium-dependent protein (it has no calcium-binding motif).

Schreiber synthesized a FK506's analogue, 506BD, and tested its binding activity against FKBP.

S. L. Schreiber *et al.*
Science **1990**, *250*, 556

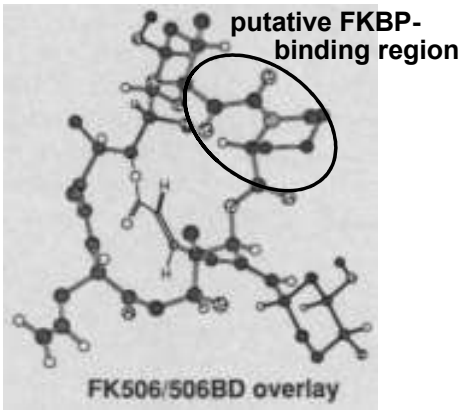


FK506



506BD

X-ray structure shows 506BD adopts a geometry similar to that of the putative binding domain in FK506.

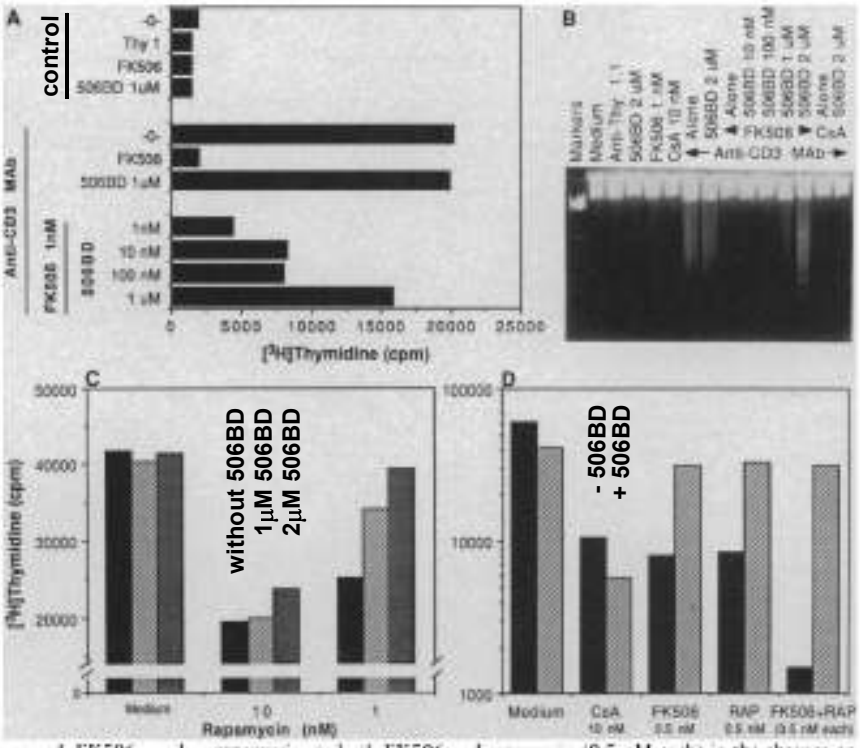


506BD actually bound to FKBP and inhibited its rotamase activity.
And 506BD could effectively displace FK506 by FKBP. (506BD's K_i value was 5 nM)

However, 506BD showed different biological effects from those of FK506.
506BD didn't prevent IL-2 production from T-cell.
Furthermore, **506BD reversed FK506's inhibition of IL-2 production.** (figure A)
Although 506BD has no effect on inihibition of T cell activation by CsA, it reversed inhibiton by rapamycin. (figure C)
(Experimental data in the next page)

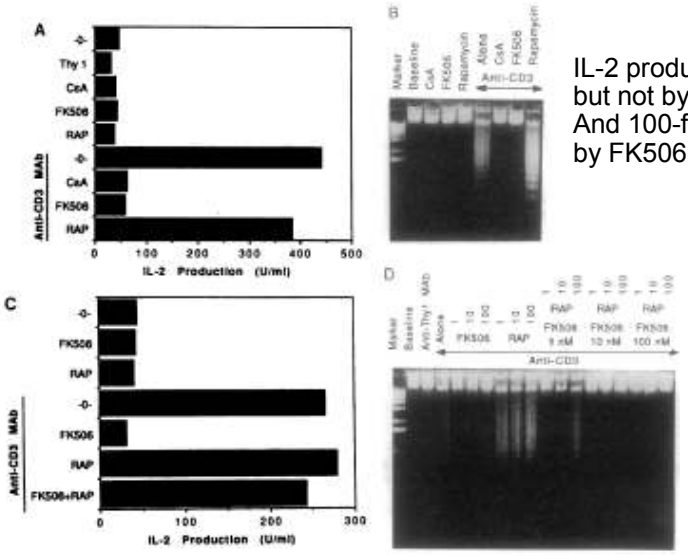
➡ **506BD works as antagonist for FK506 and rapamycin, not for CsA.**

Fig. 4. The inhibitory properties of immunophilin ligands. (**A**) 506BD reversed FK506-mediated inhibition of IL-2 production. The antigen-reactive murine T cell hybridoma 16.CD2-15.20 (*19, 20*) (10^5 cells per well) was cultured in a 24-well plate, in the absence or presence of a nonstimulatory MAb to Thy 1 or the activating MAb to murine CD3 145-2C11 (*25*), in the absence or presence of 1 nM FK506 or 506BD at the indicated concentrations. At 20 hours, culture supernatants were harvested and assayed for the presence of IL-2 by their ability to support the proliferation of an IL-2 dependent murine T cell line, CTLL-20 (*22*), as described (*4, 19*). (**B**) Whereas it has no effect alone, 506BD reversed the FK506-, but not CsA-, mediated inhibition of activation-induced cell death. Cells of the murine hybridoma 16.CD2-15.20 were cultured as described in (A). After 20 hours, DNA was extracted and electrophoresed on a 2% agarose gel as described (*4, 21*). Anti-CD3–stimulated cell death resulted in fragmentation of DNA to characteristic multimers of 180-base pair fragments. (**C**) 506BD reversed rapamycin-mediated inhibition of IL-2–dependent proliferation of CTLL-20. The IL-2–dependent T cell line CTLL-20 (5×10^3 cells per well) was cultured with human recombinant IL-2 (20 U) in the absence or presence of rapamycin or 506BD at the indicated concentrations. Proliferation was assessed by the incorporation of [^{3}H]thymidine in a 6-hour pulse after an 18-hour incubation as described (*4*). Black bar, medium; striped bar, 1 μM 506BD; gray bar, 2 μM 506BD. (**D**) 506BD effectively reversed FK506- and rapamycin-, but not CsA-, mediated inhibition of proliferation of PBMC stimulated with anti-CD3. Freshly isolated PBMC (10^5 cells per well) were stimulated with either anti-CD3 (OKT3) at a 1:40,000 dilution of ascites fluid in the presence of medium, 10 nM CsA, 0.5 nM FK506, 0.5 nM rapamycin, or both FK506 and rapamycin (0.5 nM each) in the absence or presence of 1 μM 506BD. Cells were cultured in triplicate and harvested at 72 hours after an 8-hour pulse with [^{3}H]thymidine. Black bar, medium; striped bar, 1 μM 506BD. Experiments were performed from two to four times; a representative for each is shown (*26*).



Schreiber also demonstrated that complexes between FKBP and FK506 or rapamycin might inhibit two distinct signaling-pathway in T lympocytes.

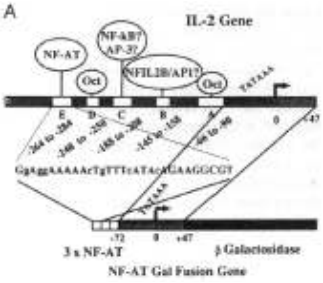
S. L. Schreiber and G. R. Crabtree *et al. Proc. Natl. Acad. Sci. USA* **1990**, *87*, 9231

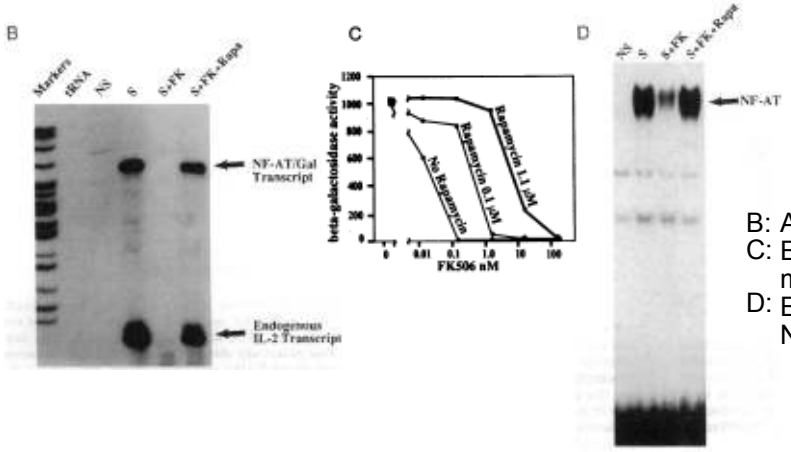


IL-2 production of T-cell was inhibited by FK506 and CsA but not by rapamycin. (figure A and B)
And 100-fold excess of rapamycin reversed the inhibition by FK506. (figure C and D)

Crabtree *et al.* had reported that a T-cell specific transcription factor (NF-AT) is controlling IL-2 gene. And NF-AT is activated after stimulation of antigen receptor of T cells.
(G. R. Crabtree *et al. Science* **1988**, *241*, 202)

Schreiber shows that FK506 inhibits a factor of pathway activating NF-AT by making model system. (figure A on right side)





B: A ribonuclease assay of transcripted mRNAs
C: Effects of FK506 & Rap. on the NF-AT activity measured as galactosidase activity
D: Electrophoreic mobility shift assay using the NF-AT binding site as probe

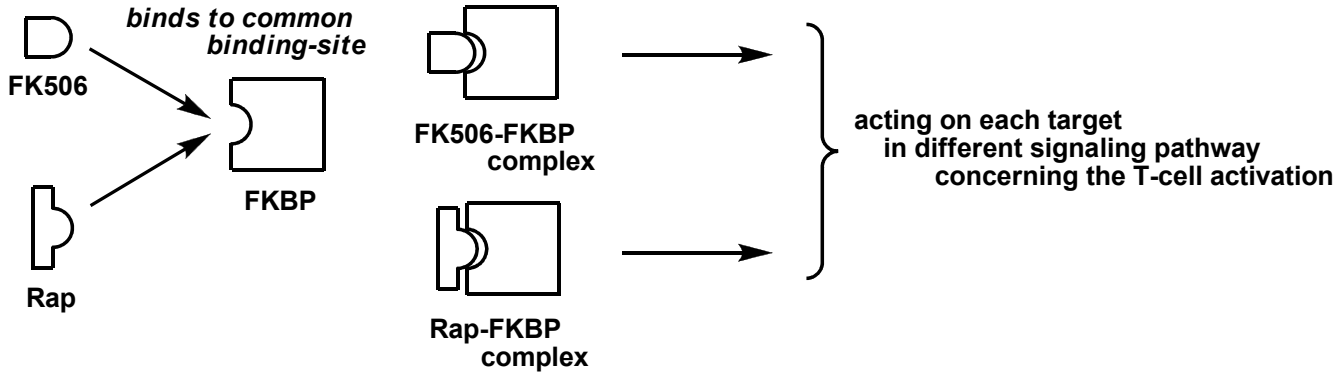
All results suggest that NF-AT activity is inhibited by FK506 and rapamycin reverses this FK506's inhibitory actions.

Considering these three series of experimental facts...

Either FK506 or rapamycin (Rap) binds to FKBP at the same binding site and works as antagonist against each other. This is supported by the fact that Rap has very a very similar structure to the FK506's putative binding site to FKBP.

FK506 apperently inhibits a T-cell activation pathway involving NF-AT, but Rap didn't. This fact suggests that Rap inhibits another step of T-cell activation.

FKBP bound to FK506 or Rap interact with different target molecule in separable pathways in T-cell activation ??



4-2. Genuine FK506's target

S. L. Schreiber *et al.*
Cell **1991**, *66*, 807

The next Schreiber's attempt is identification of the target of FK506-FKBP complex.
He used a complex between FK506 and a modified FKBP, glutathione S-transferase-FKBP12 fusion protein (GFK).
(This modified protein was prepared in an artificial genetic way and glutathione S-transferase-cyclophilin fusion protein (GCyP) was also made in the same way.)
Assay to identify the target protein was conducted by immobilizing this GFK.

glutathione binding-site and FK506 binding-site had been proved to be different.

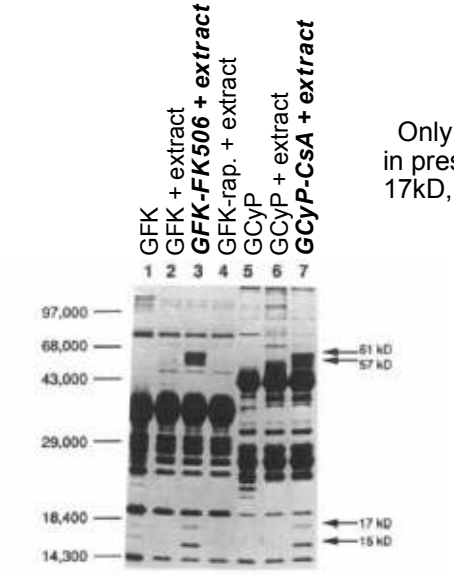


Figure 1. Detection of a Common Set of Proteins from Calf Brain Extract That Bind to GFK–FK506 and GCyP–CsA, But Not GFK, GCyP, or GFK–Rapamycin

Only when immobilized GFK or GCyP was assayed with proteins in presence of FK506 or CsA, resectively, 4 bands (61kD, 57kD, 17kD, 15kD) appeared.

→ These proteins might be the target proteins of FKBP-FK506 complex.

Experiment of Fig.2 is whether solution of immunosupressant and/or immunophilin can wash out the target protein from immmobilized-glutathione-bound GFK-FK506 complex.

This result shows only FKBP-FK506 and CyP-CsA can bind to the target proteins (=wash them out from immobilized system).
(And EGTA (Ca^{2+} ion chelator) also dissociates binding between the target protein and FKBP-FK506 complex.)

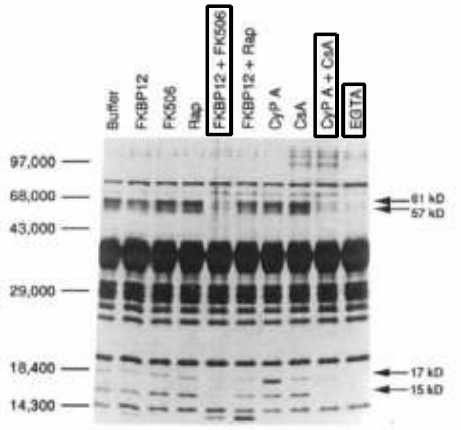
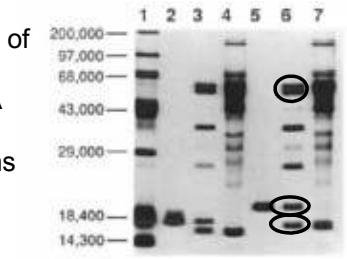


Figure 2. In Vitro Competition Experiments with Recombinant FKBP12, Cyclophilin A, Individual Drugs, Immunophilin–Drug Complexes, and EGTA

By Ca^{2+} dependency of target proteins to bind to immunophiline-immunosupressant complex, they speculate one of them is calmodulin.

And other three proteins may be subunits of calmodulin-binding protein.

Comparison in SDS-page between EGTA eluate from immobilized GFK-FK506 and authentic samples revealed the four proteins as described in figure 3.



1: Marker
2: auth. calmodulin + Ca^{2+}
3: EGTA elute + Ca^{2+}
4: auth. calcineulin + Ca^{2+}
5: auth. calmodulin + EGTA
6: EGTA elute + EGTA
7: auth. calcineulin + EGTA

Figure 3. Ca^{2+}-Dependent Gel Mobility Shift of Calmodulin, the 17 kd and 15 kd EGTA-Eluted Target Proteins, and Calcineurin B

Western blotting analysis with anti-calcineurin antibodies (fig.4) and $^{45}Ca^{2+}$ ligand blotting (fig.5) also identify these proteins.

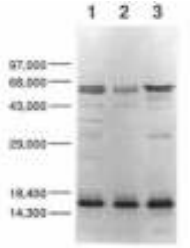


Figure 4. Western Blot Analysis of EGTA Eluate with Anti-Calcineurin Antibodies

EGTA-eluted target proteins (lane 1, 2 μg of total protein as used in Figure 3, lanes 3 and 6), calf brain calcineurin from Sigma (lane 2, 0.5 μg), and purified calf brain calcineurin (lane 3, 0.6 μg provided by Dr. C. B. Klee) were subjected to 12% SDS–PAGE and electroblotted onto nitrocellulose, which was then developed with rabbit anti-calcineurin IgG and goat anti-rabbit IgG conjugated with alkaline phosphatase.

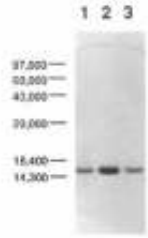


Figure 5. $^{45}Ca^{2+}$ Ligand Blotting of Calcineurin B

Lane 1, EGTA-eluted target proteins from calf thymus (2 μg of total proteins); lane 2, purified calf brain calcineurin (1 μg, provided by Dr. C. B. Klee); lane 3, calf brain calcineurin from Sigma (1 μg).

The experiments showed that **calcineurin, a Ca^{2+}- and calmodulin-dependent serine/threonine phosphatase is a common target of FKBP-FK506 and CyP-CsA complex**.
So, it's natural to consider that **calcineurin regulates phosphorylation state of some downstream target, which might be a component of signaling pathway.**

Schreiber got insights about further details of binding between FK506-FKBP complex and calcineurin.

S. L. Schreiber *et al.*
Biochemistry **1992**, *31*, 3896

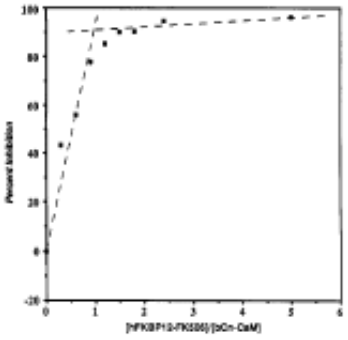


FIGURE 1: Titration of calmodulin-bound bovine brain calcineurin with hFKBP12–FK506. The titration was carried out with 300 nM calmodulin–calcineurin, 30 μM FK506, and various concentrations of the FKBP.

FK506-FKBP complex inhibits calcineurin by binding in 1:1 ratio.
And calmodulin enhances the affinity between FK506-FKBP complex and calcineurin. (Table I)
It was also revealed that FK506-FKBP and CsA-CsP are specific inhibitors for calcineurin among some protein phosphatases. (Table III)

Table I: Inhibition Constants (K_i) of Human FKBP–FK506 and Human CyP–CsA for Three Forms of Bovine Calcineurin[a]

immunophilin–ligand complex	bCn	bCn–CaM	CnA′
hFKBP12–FK506	40	32	40
hCyPA–CsA	191	33	32

[a] Abbreviations: bCn, bovine brain calcineurin; CaM, calmodulin; CnA′, 43-kDa bovine CnA fragment complexed with the B subunit of calcineurin.

Table III: Effects of Immunophilin–Ligand Complexes on Four Classes of Phosphatases

immunophilin–ligand complexes (1 μM)	% of control activity			
	PP1	PP2A	PP2C	PP2B (Cn)
FKBP12–FK506	103	117	81	5.7
CyP A–CsA	122	125	81	6.1

This graph shows inhibition rate is saturated when FKBP-FK506 complex become equal to its target, calcineulin.

4-3. The whole picture of the signaling pathway

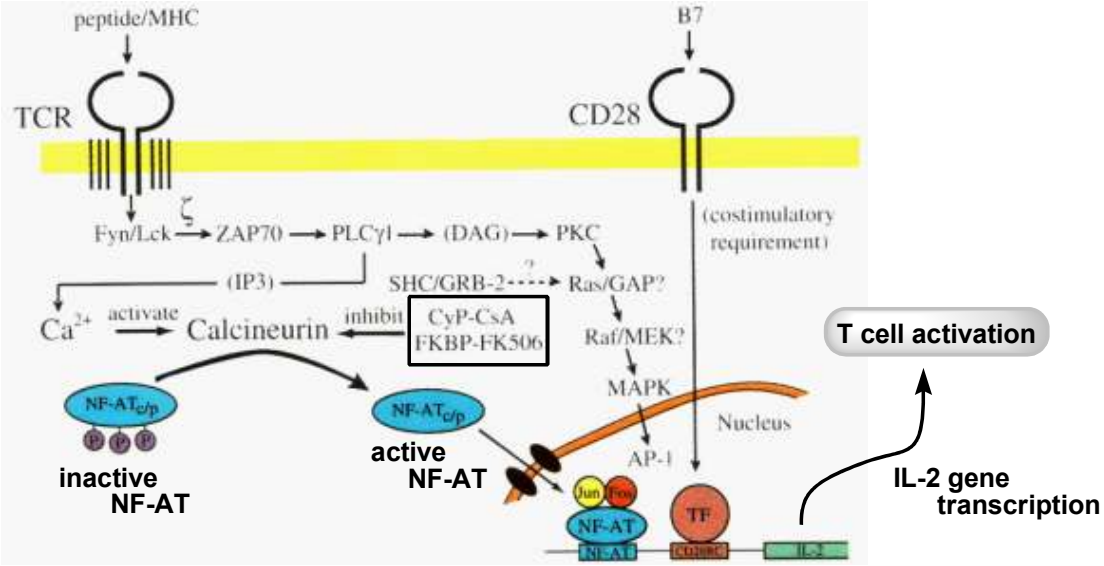
The "upstream" signaling pathway of calcineurin emanating from T cell receptor had been disclosed by other reaserchers.
And Schreiber revealed that calcineurin has a critical role for T cell activation and inhibition of calcineurin deactivates NF-AT-mediated gene transcription.

So the remaining disputable point is **how calcineurin is related to NF-AT activities.**
It was demonstrated by a research of A. Rao *et al.*

A. Rao *et al.*
J. Biol. Chem. **1993**, *268*, 3747

Calcineurin, a Ca^{2+}-regulated protein, has an ability of dephosphorylating NF-AT.
NF-AT takes an inactivated state and is localized in the cytoplasm when phosphorylated.
Contrarily, it takes an activated state and is localized in the nucleus when dephosphorylated.
Briefly, **calcineurin activates NF-AT directly.**

So Schreiber's and other researchers' studies disclosed the whole picture of the signal-transduction pathway of TCR-initiated T-cell activation. (Figure below)



Signal transduction pathway from TCR to transcription of IL-2 gene in T-cell

4-4. Structural studies of FK506-FKBP-calcineurin-calmodulin complex

Schreiber gave some insights about FK506-FKBP's binding to calcineurin from the standpoint of structure.
The FK506 binding site of FKBP was identified by X-ray crystallography and NMR spectroscopy.

S. L. Schreiber *et al. Angew. Chem. Int. Ed. Eng.* **1992**, *31*, 384

They reported some mutagenesis experiments of FKBP.
The residues which have a critical role to bind to calcineurin was identified.
(These residues was showed in the left fig. on the top of the next page.)

S. L. Schreiber *et al.*
J. Am. Chem. Soc. **1993**, *115*, 819

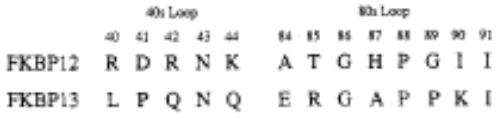


Figure 1. Amino acid sequences of FKBP12 and FKBP13 in the 40s and 80s loops.

Table I. Biochemical Properties of FKBP Mutants[a]

protein	rotamase activity[b] (10 °C) k_{cat}/K_M ($\times 10^6$ M^{-1} s^{-1})	K_i (nM) FK506[b]	K_i (nM) calcineurin[c]
FKBP12 (wt)	2.2 ± 0.2	0.4 ± 0.2	7.9 ± 3.0
FKBP13 (wt)	1.5 ± 0.3	55 ± 5	1500 ± 400
chimera **1** (40s loop exchange)	0.57 ± 0.05	0.4 ± 0.2	19 ± 2
chimera **2** (80s loop exchange)	4.2 ± 0.4	2.1 ± 0.3	580 ± 120
R40A	1.2 ± 0.4	0.1 ± 0.1	8.1 ± 2.8
R42A	1.1 ± 0.2	0.2 ± 0.1	280 ± 80
R42Q	1.3 ± 0.3	1.7 ± 0.6	850 ± 250
K44A	1.4 ± 0.2	0.1 ± 0.1	1.0 ± 0.2
K35I	1.6 ± 0.2	0.6 ± 0.2	7.8 ± 2.2
Q53A	1.8 ± 0.3	0.2 ± 0.1	5.2 ± 0.5
A84E/T85R	2.2 ± 0.2	0.6 ± 0.2	8.7 ± 1.1
G89P/I90K	1.8 ± 0.2	0.6 ± 0.2	>5000
P88V	1.5 ± 0.2	1.1 ± 0.3	16 ± 6
G89P	1.5 ± 0.3	2.7 ± 0.8	87 ± 29
I90K	3.2 ± 0.3	0.1 ± 0.1	660 ± 60
H87A	1.9 ± 0.2	1.5 ± 0.2	3.1 ± 1.8

Some analogues of FK506 had been tested for binding with FKBP and calcineurin.
The results showed C_{21}-allyl group and C_{15}-methoxy group are important to bind to calcineurin.
(These group also showed in the fig. as well.)

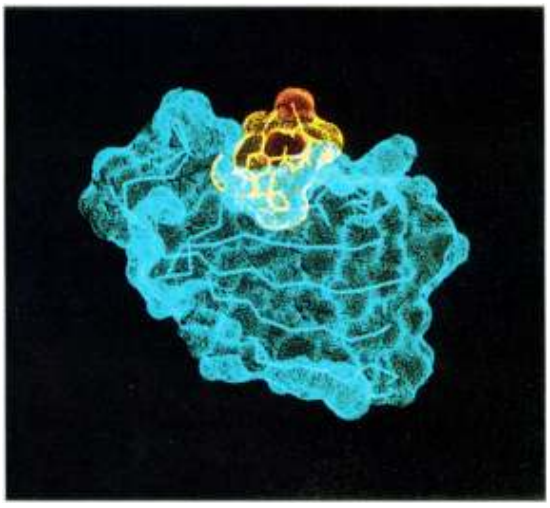
S. L. Schreiber *et al. Biochemistry* **1992**, *31*, 3896

1. FK506: R_1 = Me, R_2 = allyl;
2. FK520: R_1 = Me, R_2 = Et;
3. FK523: R_1 = Me, R_2 = Me;
4. 15-O-DeMe-FK520, R_1 = H, R_2 = Et.

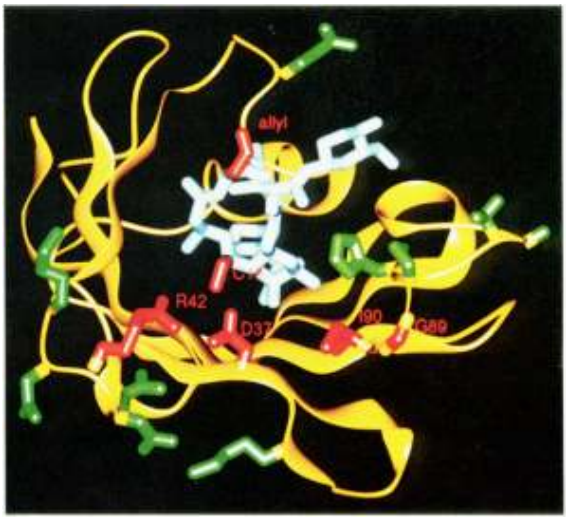
Table II: Immunophilin Binding, Calcineurin Inhibition, and Signal Transduction Inhibition by CsA and FK506 Analogues

compd no.	compound[a]	K_i (nM) for immunophilins	K_i (nM) for calcineurin	IC_{50} (nM) for NF-AT activity[b]
1	FK506	1.0	34	0.5
2	FK520	5.0	89	0.8
3	FK523	0.80	230	1.2
4	15-*O*-DeMe-FK250	15	1.6×10^3	$>8.0 \times 10^3$
5	CsA	6.0	40	3.5
6	MeBm$_2^1$-CsA	500	13	29
7	MeAla^3-CsA	9.0	$>1.0 \times 10^3$	3.2×10^3

[a] For structures of each analogue, see Figure 2. [b] As measured by NF-AT-driven β-galactosidase activity.



FKBP-FK506 complex



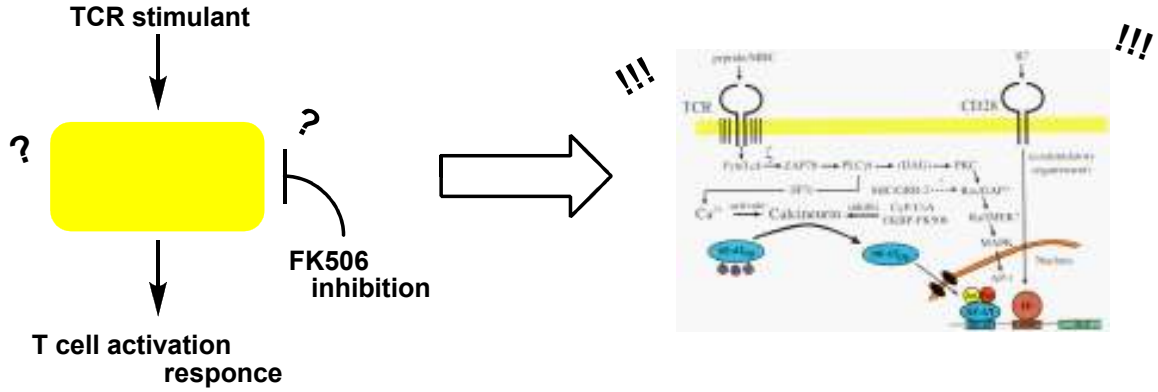
FK506 with the binding region of FKBP

The residues in FKBP and the groups in FK506 influencing the binding to calcineurin are displayed.

FK506-FKBP complex make a composite surface for binding to calcineurin.
And FK506 might act as "molecular glue" between FKBP and calcineurin.

5. Summary and Outlook

Schreiber *et al*. disclosed inside the "black box" of FK506-inhibited pathway in T cell.



Their work started from organic synthesis, then was expanded to molecular-biological studies. Since then, a series of studies like this work has attracted many chemists' attention, and became one field of science, "chemical biology", which so many scientists participate in nowadays.

What distinguished him from other chemists is that he was thinking "what the total synthesis is for". Using the synthesized molecule for testing its biological effects was a brand-new science in those days.