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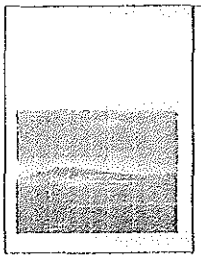

COVER Lakes and ponds on the arctic tundra with Itigaknit Mountain in the background, North Slope, Alaska. These aquatic ecosystems are continuously releasing carbon dioxide to the atmosphere. Much of the carbon originates in terrestrial environments, and accounting for this release substantially lowers the estimate of the worldwide arctic sink for atmospheric carbon dioxide. See page 298. [Photograph by George W. Kling]

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Chemistry and Biology of the Immunophilins and Their Immunosuppressive Ligands

STUART L. SCHREIBER

Cyclosporin A, FK506, and rapamycin are inhibitors of specific signal transduction pathways that lead to T lymphocyte activation. These immunosuppressive agents bind with high affinity to cytoplasmic receptors termed immunophilins (immunosuppressant binding proteins). Studies in this area have focused on the structural basis for the molecular recognition of immunosuppressants by immunophilins and the biological consequences of their interactions. Defining the biological roles of this emerging family of receptors and their ligands may illuminate the process of protein trafficking in cells and the mechanisms of signal transmission through the cytoplasm.

RESEARCH DURING THE PAST DECADE HAS CONTRIBUTED significantly to our knowledge of T lymphocyte function. The identification and functional analysis of T cell surface receptors (*1*) and nuclear transcription factors (*2*) have made these components of the signal transduction apparatus among the best understood in biology. This understanding is largely due to the use of probe reagents, such as monoclonal antibodies and radiolabeled nucleic acids, that have been developed for the study of surface and nuclear phenomena, respectively. However, the mechanisms for the transduction of signals through the cytoplasm, the "black box" of the signal transduction pathway, remain mysterious.

A family of natural products has emerged as probe reagents for cytoplasmic signaling mechanisms in the T lymphocyte. These small molecules are immunosuppressants that appear to exert their inhibitory actions distal to early membrane-associated events and proximal to nuclear processes. Studies on a family of immunosuppressant binding proteins, the immunophilins, have attempted to identify the structural requirements for high-affinity interactions between immunophilins and their immunosuppressive ligands and the biological consequences of the formation of immunophilin-ligand complexes. Although there is much to explore in this avenue of research, some general principles associated with the intermediary events of signal processing are emerging.

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The Immunosuppressants

Cyclosporin A (CsA), an inhibitor of T cell activation, is currently the favored therapeutic agent for prevention of graft rejection after organ and bone marrow transplantation, and it has been credited with initiating a revolution in clinical transplantation (*3–5*). The recently discovered compound FK506 inhibits T cell activation by mechanisms that are similar to those of CsA, but FK506 is 10 to 100 times as potent (*6*). FK506 has performed remarkably well in initial human clinical transplantation trials (*7, 8*), despite reports of toxic effects in animals (*6*). Rapamycin inhibits T cell activation at concentrations comparable to those of the structurally related FK506, yet with mechanisms that are strikingly different from those mediated by FK506, and thus CsA (*9*). Only CsA, FK506, and rapamycin have been used for the identification of members of the immunophilin class. A nonnatural ligand, 506BD (*10*), and analogs of CsA (*11–13*) have also provided insights into the inhibitory mechanisms of immunosuppressants. Many recently discovered immunosuppressive agents (*14*) with undefined mechanisms, such as

discodermolide (*15*) and deoxyspergualin (*16*), promise to reveal new facets of cytoplasmic signaling mechanisms (*17*) (Fig. 1).

The Immunophilins

The predominant CsA-binding protein in T lymphocytes is the soluble, cytosolic receptor cyclophilin (*18, 19*). Cyclophilin is an abundant and ubiquitous protein that is found in both prokaryotic and eukaryotic organisms. The major isoform of human cyclophilin has a mass of 17,737 daltons and an isoelectric point (pI) of 9.3. Two groups have independently reported that cyclophilin is identical to peptidyl-prolyl isomerase (*20, 21*), an enzyme that catalyzes the interconversion of the *cis*- and *trans*-rotamers of the peptidyl-prolyl amide bond of peptide and protein substrates, and this rotamase activity is potently inhibited by CsA.

Shortly after this discovery, the predominant FK506-binding protein in calf thymus, human spleen, and the T cell line Jurkat, termed FKBP, was isolated and characterized in two laboratories (*22, 23*). Like cyclophilin, FKBP was shown to have rotamase activity toward a peptide substrate. FK506 inhibits the rotamase activity of FKBP, but not of cyclophilin; likewise, CsA does not inhibit the rotamase activity of FKBP. The cloning (*24, 25*) and overexpression (*24*) of human recombinant FKBP and the cloning of an FKBP from *Neurospora crassa* (*26*) revealed that, despite their common enzymatic properties, FKBP and cyclophilin have dissimilar sequences. Human FKBP has a mass of 11,819 daltons and, like cyclophilin, is a basic (pI = 8.9) (*22, 24*), cytosolic protein (*27*). A prokaryotic organism, *Neisseria meningitidis*, was found to have an open reading frame that encodes an FKBP-like protein (*24*). More recently, FKBP was shown to be the predominant rapamycin-binding protein in yeast, calf thymus, and human T cells (Jurkat) (*28*). Rapamycin (dissociation constant K_d = 0.2 nM) has an even higher affinity for FKBP than does FK506 (K_d = 0.4 nM), and is also a potent inhibitor of FKBP's rotamase activity (inhibition constant K_i = 0.2 nM) (*29*).

Although cyclophilin and FKBP are the only well-characterized immunophilins, other members of this family are known to exist and are currently being investigated. For example, a CsA-binding phosphoprotein of relative molecular mass (M_r) 45,000 has been detected in Jurkat cells (*30*), and phosphoproteins of M_r 60,000 and 80,000 from this same cell line bind to both FK506 and rapamycin (*28*). The *nimA* gene of *Drosophila* (*31, 32*) and a second cyclophilin-related gene in *Saccharomyces cerevisiae* (*33*) encode proteins that show high homology to cyclophilin. Several low molecular weight, basic proteins that are retained on CsA, FK506, or rapamycin affinity matrices have also been noted (*22, 28*). Partial sequence determination of FK506- and rapamycin-binding immunophilins of M_r 30,000 and M_r 13,000 has revealed that these molecules, together with FKBP, are members of a previously unknown family of immunophilins (*34*). Questions concerning the biological relevance, the rotamase activity, and the affinity to the cognate ligands of these low-abundance immunophilins should soon be answered.

Although the exact cellular concentrations of FKBP and cyclophilin are not known, both are abundant. Saturation binding in the cytosol of Jurkat cells was reported to occur at >5 nM ditritio-FK506 (*27*). As FKBP is the predominant cytosolic receptor for drug, this measurement is largely accounted for by FKBP, and thus the cytoplasmic concentration of FKBP may approach 5 nM. The high-affinity FKBP ligands FK506 and rapamycin, however, inhibit T cell proliferation at subnanomolar concentrations (median inhibition concentration IC_{50} ~0.5 nM) (*29, 35*). Therefore, inhibition of the rotamase activity of FKBP is very likely an insufficient requirement for mediating the actions of these drugs in T lymphocytes, because only a small fraction of the enzyme would be inhibited at effective drug concentrations. This point has been confirmed by mechanistic studies of FK506 and rapamycin (see below); likewise, investigations of CsA analogs support a similar conclusion regarding the rotamase activity of cyclophilin (*12*).

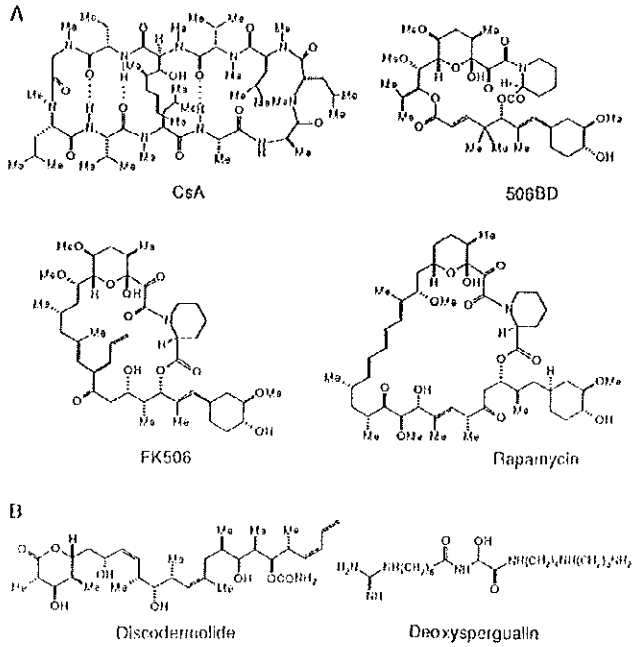

Fig. 1. Probe reagents of intracellular signaling pathways. (**A**) Recently investigated immunophilin ligands. (**B**) Immunosuppressive agents with unknown mechanisms of T cell inhibition. (Me, methyl.)

Molecular Recognition by the Immunophilins

The rotamase activity of these immunophilins and the ability of their immunosuppressive ligands to act as rotamase inhibitors provide an opportunity for exploration of the molecular basis for the high-affinity interactions that exist between them. Initial mechanistic studies of cyclophilin led to the suggestion that catalysis of the interconversion of *cis*- and *trans*-rotamers of a peptide substrate is achieved by the formation of a covalent bond to the carbonyl of the peptidyl-prolyl amide with a cysteine-derived thiol (*36*). Loss of amide resonance would be expected to lower the activation barrier to rotation about the amide C N bond. Site-directed mutagenesis of human recombinant cyclophilin allowed the systematic replacement of all four cysteine residues in cyclophilin with alanine. Because all four mutants enzymes were fully active in the rotamase and binding assays, cysteine was ruled out as a participating residue in catalysis (*37*).

Additional mechanistic studies with both cyclophilin (*38*) and FKBP (*39*) strongly suggest that these enzymes catalyze rotamer interconversion by noncovalent stabilization of the twisted amide transition state for the noncatalyzed isomerization. The amide functionality exhibits a strong preference for a planar geometry, wherein the nitrogen lone pair is in conjugation with the carbonyl π-cloud. The energy cost of the twisted amide structure (Fig. 2A) is 15 to 20 kcal (*40*). The structural basis for cyclophilin and FKBP's ability to stabilize this transition-state structure must await further structural analyses of rotamase-peptide (or inhibitor) complexes.

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