Introduction

Intra- and intercellular signal transductions are key processes that regulate cell growth, division and differentiation. The merge of natural product chemistry and cell biology has opened new possibilities for studying intracellular signaling. The logic lies in the fact that many natural products are high affinity ligands to cellular proteins involved in signal transduction.

One family of such natural products are immunosuppressive drugs cyclosporin A (CsA), FK506 and rapamycin. As shown in Figure 1, CsA is a cyclic undecapeptide. FK506 and rapamycin, which are structurally related to each other but not to CsA, are macrocyclic lactones (Figure 1). Cellular studies suggest that all three drugs suppress the immune response by blocking the activation of T lymphocytes (Schreiber, 1991; Kunz and Hall, 1993). CsA and FK506 inhibit a Ca2+-dependent signaling pathway emanating from activation of T cell receptors, and rapamycin blocks a Ca2+-independent pathway required for the proliferation of T cells upon stimulation by lymphokines such as interleukin-2 (Schreiber and Crabtree, 1992; Sigal and Dumont, 1992). In addition to blocking T cell activation, these drugs also have inhibitory effects on signaling pathways in other systems. For example, CsA and FK506 both block the Ca2+-dependent degranulation in mast cells (Hultsch et al., 1991). Rapamycin, on the other hand, has been shown to arrest yeast and some mammalian cells at the G1 phase in the cell cycle (Heitman et al., 1991; Bierer et al., 1990; Dumont et al., 1990; Price et al., 1992). These findings suggest that CsA, FK506 and rapamycin may target molecules that are common signaling components in different systems. What are the target molecules for immunosuppressive drugs?
Immunophilins in Animal Systems

Targets for Immunosuppression

To understand the molecular mechanisms of immunosuppression by CsA, FK506, and rapamycin, the cellular receptors of these drugs have been purified and characterized (reviewed by Schreiber, 1991; Fruman et al., 1994). CsA binds to a family of receptors named cyclophilins (CyPs), and FK506 and rapamycin bind to a distinct set of receptors called FKBPss (standing for FK506 and rapamycin-Binding Proteins). Cyclophilins and FKBPss are collectively referred to as immunophilins (Schreiber, 1991).

Interestingly, these receptor proteins have been subsequently shown to have peptidylproline cis-trans isomerase (PPIase or rotamase) activity (Harding et al., 1989; Fischer et al., 1989). Kinetic and structural studies have shown that the enzyme activity of both CyPs and FKBPss are competitively inhibited by binding of their cognate ligands (Kofron et al., 1991; Fesik et al., 1990; Van Duyne et al., 1991). Therefore, it was speculated that immunosuppression by these drugs is due to the loss of rotamase activity. This model was soon disproved by the finding that some drug analogs can still inhibit rotamase activity yet failed to suppress the immune response (Bierer et al., 1990). In particular, FK506 and rapamycin bind to exactly the same set of receptors but have different mode of action (Dumont et al., 1990; Bierer et al., 1990). An alternative and now widely accepted is the “active complex model”, namely, the complexes formed by immunophilins and their ligands are the functional module for immunosuppression. In support of this hypothesis, Liu et al. (1991) have shown that the FKBP12-FK506 and CyP-CsA complexes, but not their separate components, bind to and inhibit the activity of calcineurin, a Ca2+-calmodulin-dependent protein phosphatase (also called PP2B). Biochemical and cellular transfection studies have demonstrated that inhibition of calcineurin activity is necessary for the immunosuppressive effect of CsA and FK506 (Liu et al., 1992; O’Keefe et al., 1992; Clipstone and Crabtree, 1992). On the other hand, complex formed by FKBP12 and rapamycin targets a 220 kDa protein referred to as FRAP (FKBP-Rapamycin As-

sociated Protein) or RAFT1 (Rapamycin And FKBP12 Targets), a mammalian homologue of TOR1 and TOR2 in yeast that are involved in the signaling pathway leading to G1-S progression in the cell cycle (Kunz et al., 1993; Brown et al., 1994; Sabatini et al., 1994). It is speculated that FRAP/RAFT1 are required for proliferation of mammalian cells, in particular T lymphocytes where rapamycin exerts its immunosuppressive effect. Although the C-terminal sequences of these rapamycin target proteins are homologous to phosphotidylinositol (PI) kinases, the enzyme activity of TORs and FRAP/RAFT1 has yet to be established.

Immunophilins and Protein Folding

During the past several years a growing number of immunophilins have been characterized from mammalian and also from other sources ranging from bacteria and yeast to higher plants (Reviewed in Schreiber, 1991; Rosen and Schreiber, 1992; Fruman et al., 1994). The high level of conservation and ubiquitous distribution of immunophilins among divergent organisms and in almost all the subcellular compartments suggests that these proteins participate in important cellular processes.

Their attendant rotamase activity led to the suggestion that immunophilins facilitate protein folding in vivo. Evidence for this hypothesis is accumulating. In one case, CsA, a specific inhibitor for the rotamase activity of cyclophilins, delays the collagen triple helix assembly in chick embryo fibroblasts (Steimann et al., 1991). The formation of the correct form of transferrin in liver cells is also inhibited by CsA (Lodish and Kong, 1991). It is not possible, however, to know whether this correlation is due to an important role for the enzymatic activity or whether the enzyme active site is playing the role of a receptor that can bind to unfolded intermediates of protein substrates. A possible function for immunophilins in protein trafficking is suggested by studies of a cyclophilin homolog NinaA in fruit flies that is required for the transit of specific isoforms of rhodopsin from the endoplasmic reticulum (Stamnes et al., 1991). In vitro protein folding studies with carbonic anhydrase indicate that cyclophilins can function as chaperones (Freskgard et al., 1992). This possibility is further supported by
recent reports that demonstrated the heat shock-responsive expression of cyclophilin mRNAs in yeast (Sykes et al., 1993) and in a higher plant (Luan et al., 1994a).

Other intriguing findings suggest that each member of immunophilins may have more specific functions that may or may not be related to their function in protein folding processes in the cell. For example, FKBP12 associates with and modifies the activity of Ca²⁺-releasing ryanodine receptor in the striated muscle cells (Jayaraman and Marks, 1992; Brilliante and Marks, 1994). TGF-β receptor specifically interacts with FKBP12 and FK506 or rapamycin can antagonize the interaction (Wang et al., 1994). FKBP59, another cytosolic FKBP, contains at least three defined domains, a rotamase domain, a calmodulin-binding domain, and a domain interacting with the heat shock protein hsp90 in a supermolecular complex containing inactive glucocorticoid receptor (Tai et al., 1992; Lebeau et al., 1992; Peattie et al., 1992). A 40 kDa cyclophilin, CyP40, also interact with hsp90 in the steroid hormone receptor complex (Kieffer et al., 1993). It is interesting to find that the inactive steroid receptor complex contains proteins that can interact with three natural products: glucocorticoids, cyclosporin A, and macrolides (FK506 and rapamycin). FKBP25 has been localized to the nucleus and is associated with casein kinase II and nucleolin (Jin and Burakoff, 1993). CyP C, a cyclophilin with a N-terminal signal peptide, has been shown to bind to a membrane protein with homology to scavenger receptor in the macrophage (Friedman et al., 1993). Consistent with the functional implication of the CyP C receptor is the finding that activated macrophages secrete a cyclophilin with chemotactic inflammatory activity (Sherry et al., 1992). It has been shown that T cell surface has specific binding sites for CyP B (Allain et al., 1994), another membrane-associated CyP that is also partially secreted (Spik and Movva, 1991). Recently, a cyclophilin domain has been found in a 150 kDa molecule NK-TR, a component of tumor recognition complex on the surface of natural killer cells (Anderson et al., 1993). Both CyP A and CyP B are found to associate with Gag protein that is involved in the assembly and replication of human immunodeficiency virus (Luban et al., 1993). The functional significance of these molecular interactions between immunophilins and other proteins is under intensive investigation.

Immunophilins in Higher Plants

An Immunosuppressants-Sensitive Signaling Pathway in a Higher Plant

Despite the recent progresses made on the signal transduction pathways in animal and yeast systems (reviewed in Blumer and Johnson, 1994), we know very little about the molecular basis of intracellular signaling in plants. Elucidation of signaling pathways inhibited by CsA, FK506, and rapamycin in T cells has established the drugs as powerful tools for studying intracellular signal-
successively used to purify immunophilins from mammalian tissues (Fretz et al., 1991). Both CyPs and FKBP s are present in the seedling extract (Luan et al., 1993; 1994a). Two major CyPs with MW of 18 and 21 kDa (pCyP A, pCyP B) were resolved and five FKBP s are detected with MW at 12, 13, 18, 25, and 55 kDa we referred to as pFKBP12, pFKBP13, pFKBP18, and pFKBP25, pFKBP55 respectively (“p” stands for “plant” to distinguish these proteins from mammalian FKBP s).

As a first step to determine the cellular function of immunophilins in higher plants, we studied the distribution of these proteins in different plant tissues using the affinity chromatography approach. This study has revealed a unique pattern and distinct mode of regulation for immunophilin expression in higher plants in comparison with mammalian systems (Luan et al., 1994a). pFKBP13, the most abundant FKBP member in leaf tissues, was not detected in root tissues, whereas other FKBP s were present in both tissues. While the abundance of pCyP A in leaves was similar as in roots, pCyP B was expressed at a much higher level in leaf tissues than in root tissues. Subcellular localization of immunophilins in mesophyll cells showed that chloroplasts contain pFKBP13 and pCyP B but not other members, which explains the preferential expression of these two proteins in leaves over roots. The abundance of chloroplast-localized immunophilins, FKBP13 and pCyP B, was regulated by light. Although etiolated leaves produced detectable levels of pCyP B, they did not express pFKBP13. Illumination of etiolated plants dramatically increased the level of both pFKBP13 and pCyP B. The light-induced expression of pFKBP13 is closely correlated with the accumulation of chlorophyll in the leaf tissue. These findings suggest that pFKBP13 and pCyP B may play a specific role in chloroplasts (Luan et al., 1994a).

Based on an amino-terminal peptide sequence of a chloroplast-localized cyclophilin (pCyP B), we have isolated a cDNA clone encoding the preprotein of this cyclophilin (Luan et al., 1994). The deduced amino acid sequence of the cDNA starts with a putative transit sequence for chloroplast targeting. The mature pCyP B protein has rotamase activity with low substrate specificity. The enzyme activity was inhibited by CsA with an inhibition constant (K_i) of 3.9 nM. This represents the first plant rotamase characterized in a purified form. Like CyPs from mammalian cells, pCyP B, when complexed with CsA, inhibits the phosphatase activity of bovine calcineurin supporting our earlier hypothesis that CsA can bind to an endogenous CyP and form functional complex inhibitory to calcineurin-like protein phosphatase. Because pCyP B is mainly located in the chloroplast, pCyP A may be the isoform of receptors that mediate the action of CsA against calcineurin (so far all the isoforms of CyPs tested inhibit calcineurin activity in the presence of CsA). The mRNA level of pCyP B was high in leaf tissues but was not detectable in roots. Expression of the transcript in the leaf tissues was regulated by light and induced by heat shock. These findings illustrate the conserved nature of cyclophilin proteins among all of the eukaryotes and suggest that cyclophilins have a unique mode of regulation in higher plants. A chloroplast-located cyclophilin from Arabidopsis was also discovered independently by another group (Lippuner et al., 1994). By now, at least four different isoforms of cyclophilins have been characterized from Arabidopsis (Chou and Gasser, 1997), and several homologues have been isolated from other plant species as well (Marivet et al., 1994; 1995).

With the peptide sequencing information obtained for pFKBP15 (originally called FKBP18) and pFKBP12, we have isolated cDNAs for these FKBP s from both fava bean and Arabidopsis (Luan et al., 1996; Xu et al., 1998). pFKBP15 is highly homologous to FKBP13 in other organisms such as yeast and mammalian systems. It is clearly qualified for a protein that is retained in the endoplasmic reticulum (ER) because the protein starts with a signal peptide that is cleaved after maturation. In addition, the C-terminus of pFKBP15 ends with KXEL, a signal for ER retention. In Arabidopsis and rice, there are at least two isoforms of pFKBP15 indicating that pFKBP15 is encoded by a small gene family (Luan et al., 1996). pFKBP12 is most similar to FKBP12 in other organisms. However, plant FKBP12 contains critical mutation in the domain required for interaction with calcineurin and FRAP, the targets for FK506 and rapamycin. This is consistent with and provides structural basis for our previous finding that FK506 needs FKBP12 from human in order to block calcineurin function in plant guard cells (Luan et al., 1993). Together these findings have changed the general view that FKBP12 and the drug targets may have evolved along the line of drug action in eukaryotes. Another unique feature of plant FKBP12 is that they contain a disulfide bond which is critical for maintaining the structure of this protein (FKBP12 from other organisms ranging from yeast to human only contain one cysteine).

The high molecular weight FKBP, FKBP70 or FKBP73, from plants has been cloned independently by two groups (Vucich and Gasser, 1996; Blecher et al., 1996). This FKBP contains three drug-binding domains and a domain that may interact with heat-shock protein 90 (hsp90), a feature also present in FKBP59 of mammalian cells (Lebeau et al., 1992).

Summary and Perspectives

As we have discussed above, recent studies (mostly in the past 5 years) have brought immunophilins to the crossroads of immunosuppression, signal transduction, protein folding, and other cellular functions. Using immunosuppressants as probes, we have discovered a new component in the Ca^{2+} signaling pathway in higher plant cells (Luan et al., 1993). Isolation of the receptors for the immunosuppressive drugs has identified two new families of proteins in plants (Luan et al., 1993, 1994a, 1994b; Luan et al., 1996; Gasser et al., 1990; Lippuner et al., 1994; Blecher et al., 1996). Although some of
these immunophilin proteins are highly conserved among mammalian, yeast and plants, their functions in higher plants are likely to be unique especially considering the localization of two immunophils in the chloroplast. Because all immunophils possess rotamase activity and some have been shown to be chaperones, pCyP B and pFKBP13 may play a role in the refolding, sorting, and proper functioning of proteins that are imported into the chloroplast. Other immunophils from plants may play important functions in other processes such as regulation of development in response to hormones and other factors. A recent study shows that a gene encoding a FKBP73-like protein is essential for normal plant development (Vittorioso et al., 1998). Mutation of this gene causes significant defect in shoot meristem development (Vittorioso et al., 1998). Another study identified a cyclophilin-like protein that plays a role in chloroplast function (Fulgosi et al., 1998). This protein is an active rotamase but is not inhibited by CsA indicating structural difference in comparison with other cyclophilins. Further studies on plant immunophils using both molecular and genetic approaches will shed light on immunophilin function in various signaling and developmental processes.

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Literature Cited


