

V. Antifungal Drug Action and the Elucidation of Drug Targets in Fungi

Classic studies defined the mechanisms of action of the immunosuppressive drugs cyclosporine A, FK506, and rapamycin using the model yeast *Saccharomyces cerevisiae*. These studies revealed that a common set of drug targets, including cyclophilin, FKBP12, calcineurin, and Tor, underlie the action of these drugs both in fungi and in cells of the mammalian immune system. These drugs are natural products with potent antimicrobial activity that can be employed to define fungal virulence determinants and possibly also as novel therapeutics.

We have focused on the immunosuppressive drugs, cyclosporin (CsA), FK506, and rapamycin, which suppress the immune system by blocking activation of T-lymphocytes. CsA, FK506, and rapamycin are widely used to treat and prevent graft rejection in organ transplant recipients. These compounds are all natural products of soil microorganisms and play a role in nature distinct from immunosuppression, likely as toxins to inhibit growth of competing microorganisms. Based on this hypothesis, we have analyzed in detail the mechanisms of drug action in *S. cerevisiae*. These studies reveal signaling cascades targeted by these drugs are conserved from yeast and pathogenic fungi to humans.

Each of these inhibitory molecules diffuses into the cell and associates with a binding protein, CsA with cyclophilin and FK506 and rapamycin with FKBP. The cyclophilin and FKBP proteins are enzymes that catalyze a rate limiting step in protein folding. The drugs bind to and inhibit the enzyme active sites, but this is not how cell function is disrupted. Yeast and fungal cells missing the cyclophilin or FKBP proteins are viable and completely resistant to these drugs. Thus, these compounds do not kill the cell by inhibiting the binding proteins. Instead, the protein-drug complexes are the active agents, and these complexes bind and inhibit signaling molecules. The target of the cyclophilin-CsA and FKBP-FK506 complexes is calcineurin, a highly conserved calcium sensing protein phosphatase.

Our studies now address the normal cellular functions of these drug targets. We discovered that calcineurin is required for mating, filamentation, and virulence in *C. neoformans* and are identifying the calcineurin substrates and other elements of the signaling cascades regulating these processes. A calcineurin binding protein has been identified in *C. neoformans* that is conserved in yeast and humans and may represent a calcineurin effector. This calcineurin binding protein is the first gene in the Down's syndrome critical region on human chromosome 21. Both calcineurin and the calcineurin binding protein DSCR1 are highly expressed in the heart and the brain, two tissues prominently effected in Down's Syndrome patients, suggesting DSCR1 overexpression and perturbations in calcineurin signaling could underlie some of the clinical manifestations of this disorder.

The role of calcineurin in promoting fungal virulence has been extended to *Candida albicans* and *Aspergillus fumigatus* (in collaboration with John Perfect and Bill Steinbach). Importantly, calcineurin's role in virulence is unique in each species, and serves to promote survival at mammalian body temperature in *C. neoformans*, survival in serum in *C. albicans*, and ability to engage in filamentous growth in *A. fumigatus*. We have discovered that calcineurin is also required for *C. albicans* to survive membrane stress exerted by the azole class of antifungal drugs. As a consequence, calcineurin mutation or inhibition renders azoles fungicidal. Drug combinations that target calcineurin or Hsp90 in combination with azoles are being explored in a variety of pre-clinical models for *Candida* and other fungal infections, including systemic, ocular, and cutaneous infections.

In collaboration with Steve Hanes in Albany, we have found the functions of the cyclophilin A prolyl isomerase overlap with a second prolyl isomerase, the parvulin homolog Ess1/Pin1. We have identified targets of the Ess1 and cyclophilin A proteins as the CTD domain of RNA polymerase II and a histone deacetylase complex. We have found that cyclophilin A is localized to the nucleus in yeast cells and governs the meiotic gene program to promote efficient sporulation, and cyclophilin A prolyl-isomerase enzymatic activity is necessary for this role in meiosis. We have confirmed that cyclophilin A physically associates with the Set3C histone deacetylase and analyzed in detail the structure of this protein-protein complex. Genetic studies support models in which cyclophilin A controls meiosis via both Set3C and an additional unknown target. Our findings reveal a novel nuclear role for cyclophilin A in governing the transcriptional program required for the mitotic to meiotic developmental switch in *S. cerevisiae*. In parallel, we have shown that two closely related cyclophilin A homologs, Cpa1 and Cpa2, are expressed in *C. neoformans*. The Cpa1 and Cpa2 cyclophilin A proteins mediate cyclosporin A antifungal action and have a shared function that is important for cell growth. These findings provide a second genetically tractable model system in which to explore the in vivo functions of this conserved but enigmatic family of protein folding enzymes.

In summary, our studies began with the unusual properties of natural product toxins and have now led to the identification of conserved target proteins whose diverse functions in cell growth and signal transduction remain to be elucidated. Much of experimental biology has been based on the premise that studies of model organisms, including bacteria, yeast, insects, and worms, would reveal conserved principles that govern how all organisms function. Our studies support this view and suggest further studies of model organisms will continue to contribute much to our understanding of the molecular basis of life.