Structure-function, trafficking, and drug discovery studies of falcipain cysteine proteases of the malaria parasite *Plasmodium falciparum*

Puran S. Sijwali[1], Kailash C. Pandey[1], Shoba Subramanian[1], Jiri Gut[1], Jennifer Legac[1], Iain Kerr[2], Linda Briner[2], Jose M. Coterón[3], Santiago Ferrer[3], and Philip J. Rosenthal[1]

Departments of Medicine[1] and Cellular & Molecular Pharmacology[2], University of California, San Francisco, CA, USA and GlaxoSmithKline, Tres Cantos, Spain[3]

Erythrocytic malaria parasites degrade hemoglobin in an acidic food vacuole to acquire amino acids and maintain homeostasis. Hemoglobin hydrolysis appears to be a cooperative process involving multiple proteases, including the papain-family cysteine proteases falcipain-2 and falcipain-3. Evidence supporting critical roles for these enzymes include biochemical studies showing hydrolysis of native hemoglobin at multiple sites, inhibitor studies in which specific inhibitors block hemoglobin hydrolysis, and gene disruption studies in which parasites with knockout of falcipain-2 had altered hemoglobin hydrolysis and those with knockout of falcipain-3 were not viable. We have determined the roles of multiple domains of falcipain-2 that are unique compared to other characterized cysteine proteases. Within the mature protease, an N-terminal extension mediates protein folding in a manner unique to so-characterized papain-family proteases and a C-terminal insertion mediates binding to hemoglobin to facilitate hydrolysis of this substrate. Within the protease prodomain, domains both up- and down-stream from a membrane spanning domain mediate trafficking to the food vacuole; such bipartite-motif directed organellar trafficking has not previously been described. Other, more down-stream regions of the prodomain are required for autoinhibition of falcipain-2. Characterization of unique functions of falcipain domains highlights potential new avenues for inhibition, and thus antimalarial chemotherapy. Studies of active site-directed inhibitors have identified small molecules with good drug properties and potent inhibition of falcipain-2 and falcipain-3. Optimal compounds also block development of cultured malaria parasites at low nanomolar concentrations and, using a new animal model, cure mice infected with *P. falciparum*. To facilitate drug discovery, we have solved four falcipain structures: falcipain-2 complexed with protein (cystatin, chagasin) and small molecule (E-64) inhibitors, and falcipain-3 complexed with leupeptin. Improved understanding of the inhibition and structural features of falcipains should facilitate drug discovery directed toward the identification of specific falcipain inhibitors as new antimalarial drugs.