# Molecular Characterization and Ultrastructural Localization of *Plasmodium falciparum* HSP 60

Hisashi FUJIOKA, Ashis DAS*, Chiang SYIN**, Hong ZHENG*, Neil GOLDMAN**, Masamichi AIKAWA*** and Nirbhay KUMAR*

*Institute of Pathology, Case Western Reserve University, USA*
**Department of Molecular and Immunology, Johns Hopkins School of Public Health,*
***Laboratory of Parasitic Biology and Biochemistry, Office of Vaccine Research and Review, Center for Biologies Evaluation and Research, Food and Drug Administration,*
****The Institute of Medical Sciences, Tokai University, Kanagawa, Japan*

The response to disturbances in physiological conditions in all organisms is governed by finely regulated cellular adaptive processes. One such response involves regulation of the expression of stress proteins (also known as heat shock proteins (Hsp)). Members of various Hsp families are highly conserved proteins which are phylogenetically widely represented. Genes for members of the Hsp 70, 90 and 60 families have been cloned in the human malaria parasite *Plasmodium falciparum*. In this study, we have cloned and expressed the *P. falciparum* Hsp 60 in *E. coli*. The sequence analysis identified a previously unknown intron of 257bp beginning after the nucleotide 142 in the coding sequence. Antisera raised against the recombinant *Pf*Hsp 60 was employed in immunoprecipitation studies with biosynthetically-labeled parasite extracts to investigate regulation of expression of *Pf*Hsp 60 at various temperatures. In contrast to a 3-4 fold accumulation of *Pf*Hsp 60 transcripts in heat shocked parasites (37℃ to 40℃), the expression of *Pf*Hsp 60 was not induced in the blood stages of *P. falciparum*. On the other hand, the effect of the heat induction of *Pf*Hsp 70 was seen both at the level of specific mRNA and protein. In these studies we also observed co-immunoprecipitation of a number of other cellular proteins suggesting possible interaction with *Pf*Hsp 60. Immunofluorescence assay (IFA) indicated the presence of *Pf*Hsp 60 in the cytoplasm of all the various stages of the parasite. The trophozoite of *P. falciparum* appeared to have mitochondria with few cristae or acristate mitochondria. Immunoelectron microscopic analysis revealed the distinct mitochondrial localization of *Pf*Hsp 60 in the asexual stage of *P. falciparum*. Moreover, a band pattern of localization of the *Pf*Hsp 60 also indicated it's presence in certain compartments in the mitochondria. *Pf*Hsp 60 was also localized in the mitochondria of the gametocyte, sporozoite, and EE stage of *P. falciparum*, but not in the mitochondria of human liver or, human hepatoma cells, and the mosquito salivary gland cells. The constitutive expression of *Pf*Hsp 60 in different parasite stages occurring in vertebrate and invertebrate hosts suggested a biologically significant role for the presence of the protein. One such role for *Pf*Hsp 60 could be its chaperonin-like activity via interaction with other cellular proteins.

This work was supported by NIH grant AI 31589 (NK) and in part by grants to MA from the U. S. Agency for International Development (DPE-936-6001-29), the U. S. Public Health Service/National Institutes of Health AI-35827 and a grant-in-aid for scientific research on priority areas from the Ministry of Education, Science, Sports and Culture of Japan.

Hisashi FUJIOKA, Institute of Pathology, Case Western Reserve University, 2085 Adelbert Road, Cleveland Ohio 44106, USA