Plasmodium falciparum cysteine protease falcipain 3: a potential enzyme for proteolytic processing of histone acetyltransferase PfGCN5

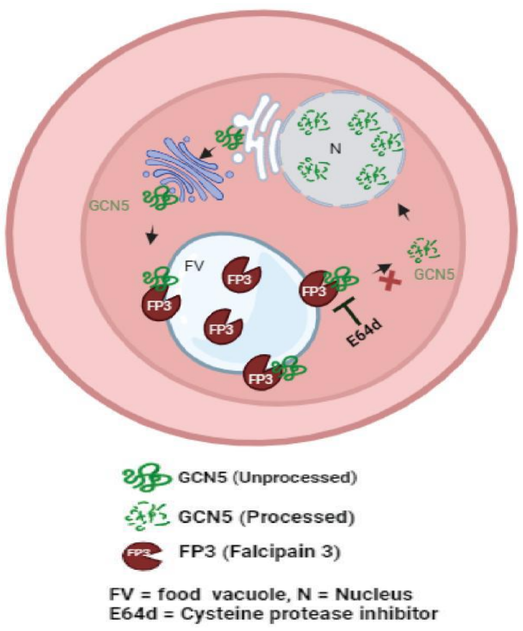
Poonam Nagar[1,2] Krishanu Bhowmick[1,3] Aishwarya Chawla[1] Md. Zubbair Malik[4,5] Naidu Subbarao[4] Inderjeet Kaur[6,7] Suman Kumar Dhar*[1,8]

1Special Centre for Molecular Medicine, Jawaharlal Nehru University, New Delhi, India
2International Centre for Genetic Engineering and Biotechnology, New Delhi
3The Institute for Bioelectronic Medicine, The Feinstein Institutes for Medical Research, Northwell Health, Manhasset, New York, USA
4School of Computational and Integrative Sciences, Jawaharlal Nehru University, New Delhi, India
5Department of Genetics and Bioinformatics, Dasman Diabetes Institute, Dasman, Kuwait
6International Centre for Genetic Engineering and Biotechnology, New Delhi, India
7Department of Biotechnology, Central University of Haryana, Mahendergarh, Haryana, India
8TERI School of Advanced Studies, Vasant Kunj, New Delhi, India

ABSTRACT

In spite of 150 years of studying malaria, the malarial parasite, *Plasmodium*, still perplexes researchers. One of the methods by which the parasite manages its gene expression is epigenetic regulation, the champion of which is PfGCN5, an essential enzyme responsible for acetylating histone proteins. PfGCN5 is a ~170 kDa chromatin-remodeling enzyme that harbors the conserved bromodomain and acetyltransferase domain situated in its C-terminus.

PfGCN5 is proteolytically processed but the specific protease involved in this process still remains elusive. Identification of PfGCN5 interacting proteins through immunoprecipitation followed by LC-MS/MS analysis revealed the presence of food vacuolar proteins, like cysteine protease Falcipain 3 (FP3), apart from the typical members of the PfGCN5 complex. Direct interaction between FP3 and PfGCN5 was validated by in vitro pull-down assay and immunoprecipitation assay.



Subsequently, use of cysteine protease inhibitor E64d led to the inhibition of processing of PfGCN5 with concomitant enrichment and co-localization of PfGCN5 and FP3 around the food vacuole as evidenced by confocal microscopy and Electron Microscopy. The proteolytic cleavage of the nuclear protein PfGCN5 by food vacuolar protease FP3 is exceptional and atypical in eukaryotic organisms. Targeting FP3, involved in processing of PfGCN5 could provide a novel approach for drug development.

RESULTS

1. Generation of 3' GFP-glmS integration parasite line at PfGCN5 genomic locus

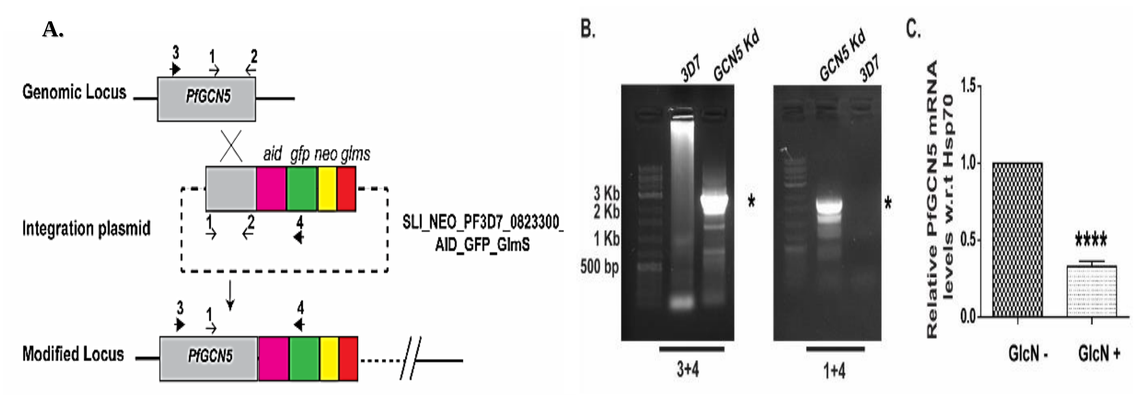


Figure 1. (A) The schematic for the integration strategy shows the endogenous tagging of *PfGCN5* at its genomic locus. (B&C) Diagnostic PCR was performed using specific primer sets (indicated by numbers over arrows) to verify integration at the *Pfgcn5* genomic locus in the obtained transgenic parasites.

2. Live Cell Imaging & Immuno EM depicting the nuclear localization of PfGCN5 within the parasite

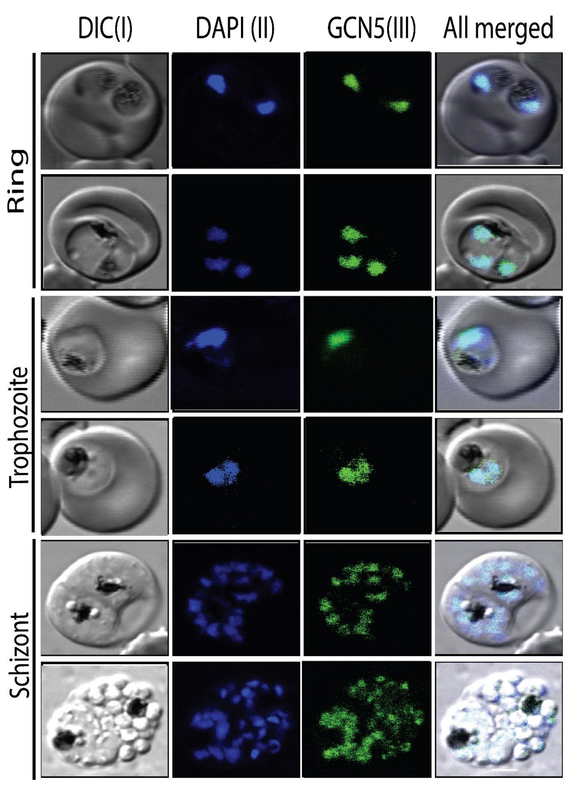


Figure 2. (a) Live cell confocal microscopy images shows the distribution and subcellular localization of the GFP-tagged PfGCN5 protein (indicated in green) within the parasites.

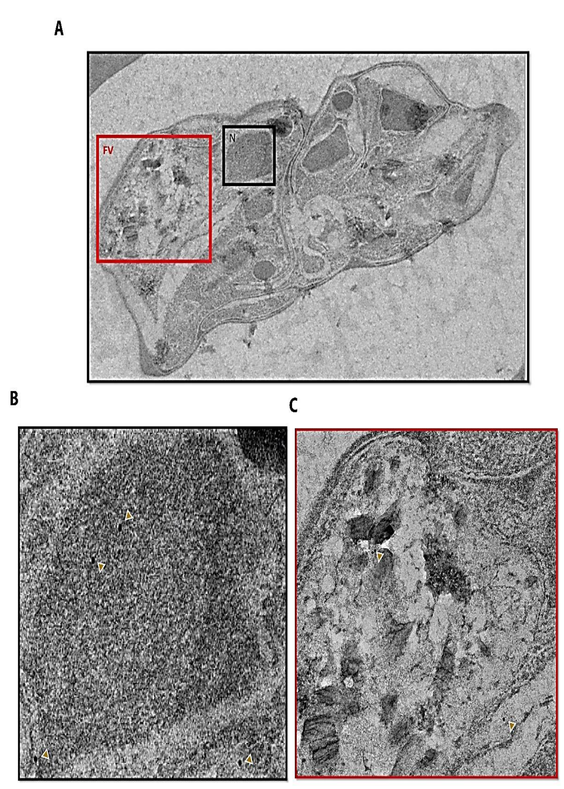


Figure 2. (b) Immuno-electron microscopy images demonstrating localization of PfGCN5 in GFP-PfGCN5 parasite line labelled with antibodies against GFP followed by secondary antibodies conjugated with colloidal gold particle (18 nm).

3. Falcipian 3 is an interacting partner of PfGCN5 in *Plasmodium falciparum*

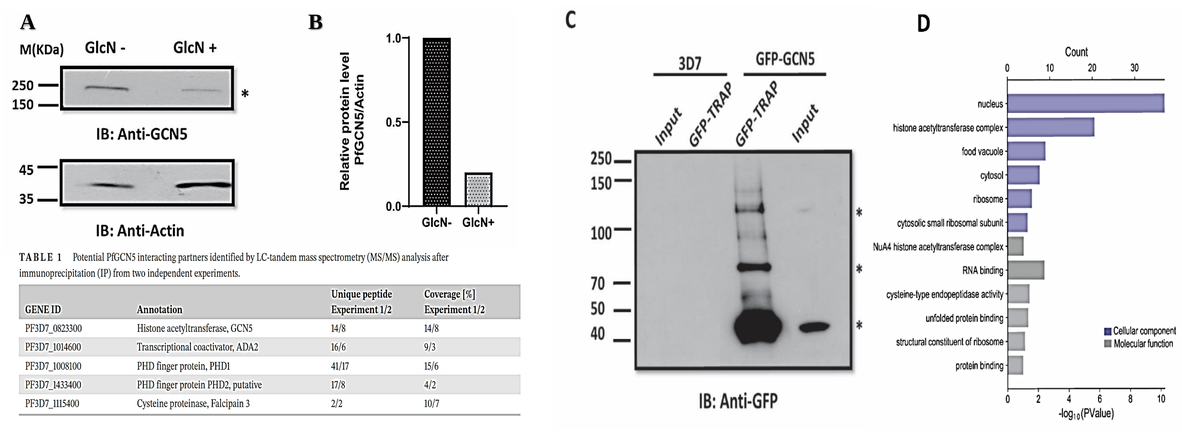


TABLE 1 Potential PfGCN5 interacting partners identified by LC-tandem mass spectrometry (MS/MS) analysis after immunoprecipitation (IP) from two independent experiments.

GENE ID	Annotation	Unique peptide Experiment 1/2	Coverage [%] Experiment 1/2
PF3D7_0823300	Histone acetyltransferase, GCN5	14/8	14/8
PF3D7_1014600	Transcriptional coactivator, ADA2	16/6	9/3
PF3D7_1008100	PHD finger protein, PHD1	41/17	15/6
PF3D7_1433400	PHD finger protein PHD2, putative	17/8	4/2
PF3D7_1115400	Cysteine proteinase, Falcipain 3	2/2	10/7

Figure 3. Immunoprecipitation (IP) of PfGCN5 using GFP-PfGCN5 parasite line. (A) Western blot analysis employing antibodies against GCN5 (B) The graph depicts the relative protein expression of GCN5/Actin in GlcN- and GlcN+ parasites (C) IP of endogenous GFP-PfGCN5 protein from parasite lysate followed by LC- MS/MS analysis. (D) Bar plot showing enriched cellular component and molecular function of protein identified after IP of GFP-PfGCN5.

4. PfGCN5 interacts with cysteine protease Falcipain 3 *in vitro* and *in vivo*

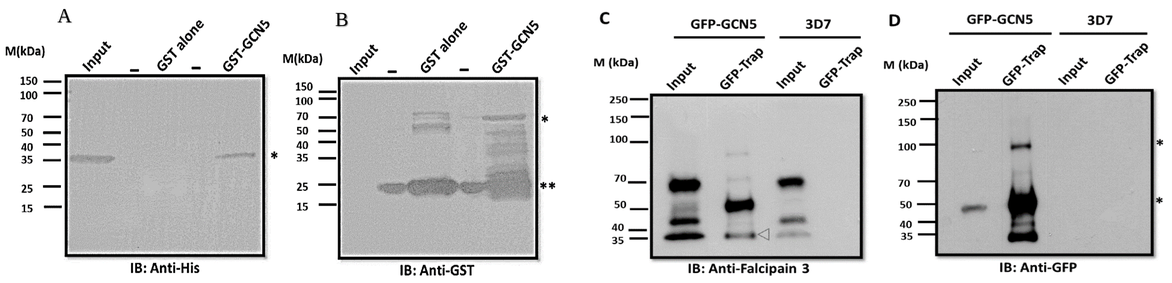


Figure 4. Validating interaction between Falcipain 3 (FP3) and PfGCN5. (A) *In vitro* GST pull-down assay followed by western blot analysis. (B) Loading of the GST tagged proteins was tested using antibodies against GST. (C) Immunoprecipitation (IP) followed by western blot analysis using specific antibodies against (C) FP3 and (D) GFP exhibits successful co-IP of GFP-PfGCN5 and FP3 within the parasites.

5. Inhibition of FP3 stabilizes PfGCN5 full-length protein in the vicinity of Food Vacuole

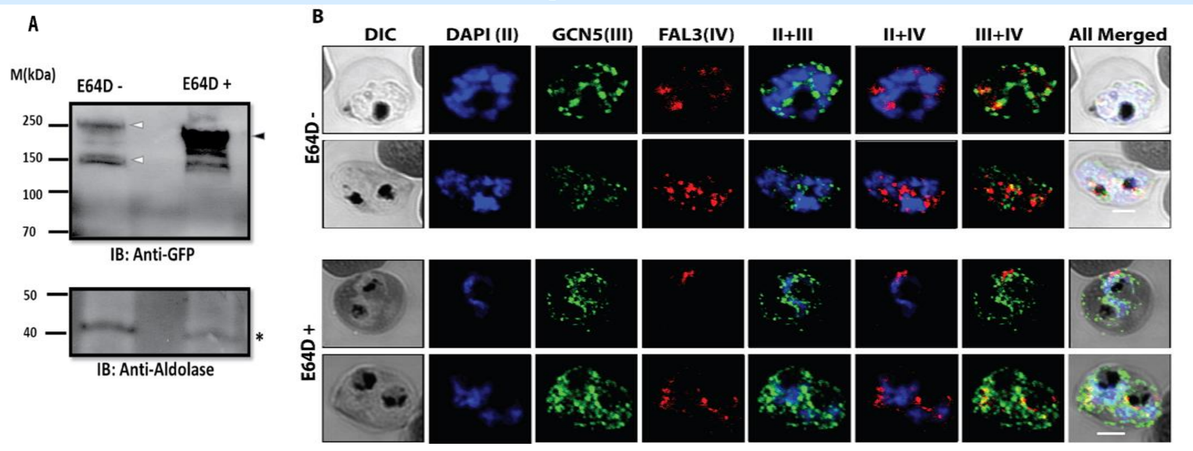


Figure 5. Cysteine protease Falcipain 3 (FP3)-mediated processing of PfGCN5. (A) Western blot analysis was done using lysate prepared from untreated and E64d-treated parasites. (B) Co-immunofluorescence (Co-IFA) analysis was carried out using GFP-PfGCN5 expressing parasite line where untreated and E64d (10 μM)-treated parasites were processed in the presence of antibodies against GFP and FP3.

6. PfGCN5 gets processed by FP3 around the Food vacuole

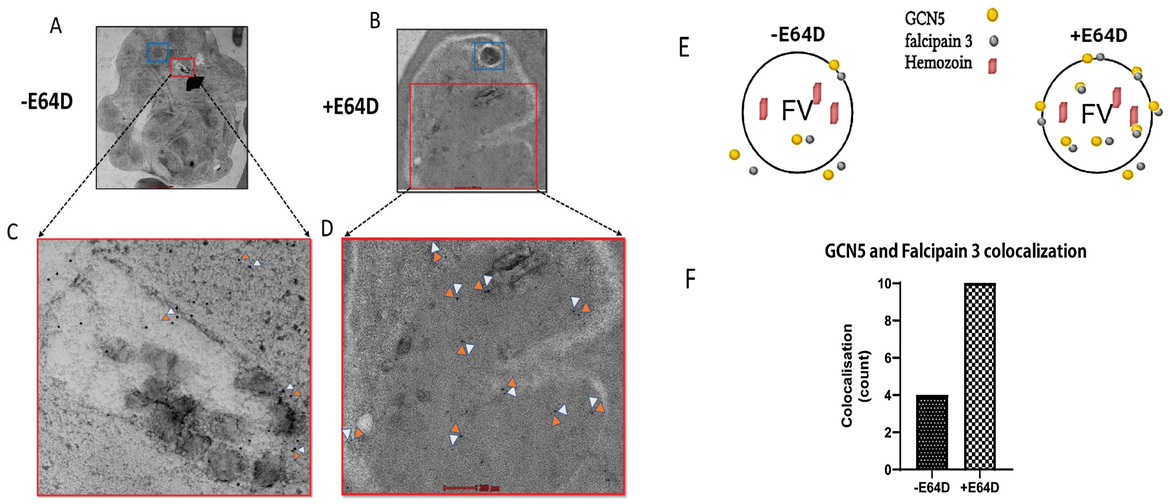


Figure 6. Immuno-EM showing colocalization of Falcipain 3 (FP3) and PfGCN5 in the parasite. (A and B) Immuno-electron microscopy (Immuno-EM) images were used to observe the *in vivo* co-localization of PfGCN5 and FP3 protein. (C and D) The selected FV region (Red square) is shown at the higher magnification. (E) A schematic showing the colocalization of gold particles. (F) Bar graph showing the quantitative analysis of co-localization at FV from the electron micrographs.

ACKNOWLEDGEMENT

1. We would like to acknowledge DPRF, DST SERB for funding and UGC for fellowship.
2. We would like to acknowledge DBT for BioCARe Women Scientist Post Doctoral Fellowship.
3. Dr. Richard Bartfai, Germany for Providing us GFP-GCN5 construct.
4. FP3 antibody was a gift from Dr. Pawan Malhotra, ICGEB, Delhi.
5. His_6-FP3 recombinant expression construct was provided by Dr. Puran Sijwali, CCMB, Hyderabad.

poonam.nagar@icgeb.res.in
linkedin.com/in/dr-poonam-nagar-952226115

Department of Biotechnology, Government of India
Anusandhan National Research Foundation
EMBL Conference BioMalPar XXII: biology and pathology of the malaria parasite