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Identification of R2TP complex of *Leishmania donovani* and *Plasmodium falciparum* using genome wide in-silico analysis

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Recently discovered R2TP complex is an important multiprotein complex involved in multiple cellular process like snoRNP biogenesis, PIKK signaling, RNA polymerase II assembly and apoptosis. Within R2TP complex, Pih1 tightly interacts with Rvb1/Rvb2 and with Tah1 to form R2TP macromolecular complex. R2TP complex further interacts with Hsp90 to form R2TP-Hsp90 complex, which has been found critical in many cellular process. The genome wide screening of *Leishmania donovani* and *Plasmodium falciparum* led to the identification of RuvB like1, RuvB like 2, Pih1, and Tah1. Therefore, we speculate that this complex is also important for these parasites as in the yeast. The detailed analysis of crucial components of R2TP complex, Ld-RuvB like 1, and Ld-RuvB like 2, revealed the presence of characteristic motifs like DNA binding motif and ATPase motifs. Hsp90 is also reported from *Leishmania donovani* and *Plasmodium falciparum* suggesting that the R2TP complex further interacts with Hsp90 to form R2TP-Hsp90 complex. Recently it has been discovered that RuvB like proteins are overexpressed in many cancers and their ATPase activity is crucial for cancer cell proliferation and the human RuvBs have been proposed as suitable drug target for cancer. Similarly one of the *Plasmodium falciparum* RuvB like protein (PfRuvB3) has been found to be specific to the stage where nuclear division led multiplication of parasite take place. Considering all these it seems that the R2TP complex may be playing some critical role both in the cancer cell proliferation in human and rapid multiplication of the parasites *Leishmania donovani* and *Plasmodium falciparum*.

Introduction

R2TP complex and its involvement in cellular processes

During investigation of physical and genetic interactome of heat shock protein 90 (Hsp90) in *Saccharomyces cerevisiae* several putative interacting proteins were identified, among them 2 protein Pih1 and Tah1 was named after their characteristic.[1] Protein interacting with Hsp90 was termed as Pih1 (also known as Nop17) while TPR containing protein associated with Hsp90 was termed as Tah1. Pih1 is well known to interact with Rvb1, Rvb2, and Tah1 and form R2TP complex.[2] Later, human R2TP complex was purified from human cells containing similar component like RuvBL1/RuvBL2, PIH1D1, and RPAP3.[3] Thus it seems that both yeast and human R2TP complexes are conserved while other mammalian R2TP complex also contains some components of the perfoldin complex.[4] Tah1 of R2TP complex is known to interact with hsp90 and form R2TP-Hsp90 complex.[2]

The components of R2TP multiprotein complex of yeast and human have been studied by different groups and their importance has been explored in many cellular processes (**Fig. 1**). RuvB1 and RuvB2 are the 2 proteins, which have been previously identified as chromatin remodeling factors later these proteins were found to be involved in the multiple cellular process like transcription, telomerase core complex assembly, cell cycle progression, apoptosis, snoRNPs biogenesis, PIKK mediated signaling, RNA polymerase II assembly, as well as in the mitosis.[5-7] Recently human RuvB1 and RuvB2 are in the focus because of their involvement in many human cancers[8] like hepatocellular carcinomas,[9] renal and gastric carcinomas.[10] The biochemical characterization reports on RuvB like proteins established that these are active ATPase and many reports have also shown their helicase activity.[6] Due to the presence of ATPase activity and involvement in multiple cellular processes these proteins have been categorized as AAA+ proteins (ATPases associated with multiple cellular processes).

Pih1 is an unstable protein however stabilizes itself after interaction with Hsp90 and Tah1.[2] Pih1 interacts with snoRNPs factor Nop1/fibrillarin, Nop58, Tel2, Monad and play crucial role in snoRNPs biogenesis and PIKK signaling.[11]

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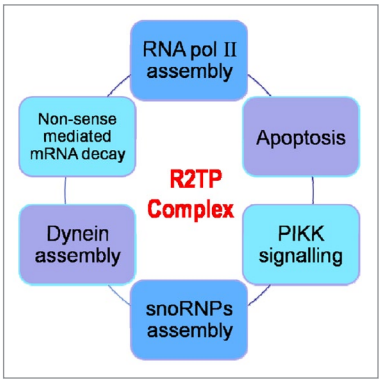

Figure 1. Schematic diagram of R2TP complex involvement in diverse cellular activities. The schematic of R2TP complex involvement in various activities was prepared on the basis of reported role in yeast and human. R2TP complex is involved in the diverse cellular activities shown in the figure.

Tah1 is characterized as the cofactor of Hsp90 and enhances the activity of Hsp90 protein.[1] Human counterpart of Tah1 is known as RPAP3 which is almost 6 times bigger in terms of number of amino acid as well as in number of TPR repeat. Yeast Tah1 contains 2 TPR repeats while RPAP3 contains 6 TPR repeats.

R2TP complex has been found to play an important role in many processes like RNA polymerase-II assembly, snoRNPs assembly, PIKK signaling, and in the apoptosis.[11] R2TP-Hsp90 complex together with Perfoldin like complex interacts with RNA polymerase-II and was found to be crucial for the assembly of the RNA polymerase-II.[4] In yeast Pih1 deletion led disruption of R2TP complex resulted into decreased snoRNAs of box C/D.[2] Human RuvB1 and RuvB2 interact with almost all core box C/D snoRNPs factor and PIH1D1 directly interacts with Nop1, Nop58, and Nop56. RuvBs have been crucial for the box C/D and box H/ACA snoRNA.[12,13] The depletion of human RuvBL1 and RuvBL2 showed a decreased level of mature box C/D snoRNA,[14] thus providing the evidence of R2TP complex involvement in snoRNP assembly and biogenesis. Recently it has been discovered that R2TP complexes are also involved in the PIKK (phosphatidylinositol-3 kinase related protein kinase) signaling pathway.[15] RuvBL1 and RuvBL2 interact with all the 6 PIKKs (ATM, ATR, DNA-PKcs, mTOR, SMG-1, and TRRAP) in mammals. RuvBL1/RuvBL2 regulates the downstream signaling via phosphorylation led activation of ATM, ATR, mTOR, SMG-1: Chk2, Chk1, p70S6K, and UPf1 respectively.[11] SMG-1 is critical component of the mRNA surveillance complex known to be involved in the non-sense mediated mRNA decay.[11,15] Furthermore, R2TP-Hsp90 complex is essential player in the stability and assembly of PIKKs. Human R2TP complex component like RuvBL2, RPAP3, and PIH1D1 have been identified as the interacting partners of monad (a subunit of Perfoldin like complex) that is involved in the apoptosis.[16]

In this article, we have extensively investigated the *Leishmania donovani* and *Plasmodium falciparum* genome for the putative components of the R2TP complex using bioinformatics approaches and further characteristic domain identification and modeling of the proteins was also done. The phylogenetic analysis was also performed for every component of the putative R2TP complex of *L. donovani* and *P. falciparum*. The genome wide screening of the *L. donovani* and *P. falciparum* led to the identification of all the components of the complex such as RuvB like1, RuvB like 2, Pih1, and Tah1.

Results and Discussion

Identification of the R2TP complex component of *Leishmania donovani*

In order to explore the components of R2TP complex in *Leishmania donovani*, we used amino acid sequence of each component of the yeast and human R2TP complex as a basic tool to study. BLAST-P analysis of yeast RuvB like protein sequences in the *Leishmania donovani* genome database showed many hit and their further screening on the basis of high score revealed that 2 RuvB like proteins are present in the *Leishmania donovani* genome and their genedb numbers are LdBPK_343280.1 and LdBPK_342440.1. Amino acid sequence of both RuvB like proteins was retrieved and protein BLAST analysis with yeast as well as human genome shows that both have considerable homology with human Pontin (Hs-RuvBL1) and Reptin (Hs-RuvBL2) as well as with yeast Rvb1 and Rvb2. Further analysis of these sequences with InterProScan showed that these proteins contain AAA+ domain/RuvB like domain (**Fig. 2**), which is characteristic of the RuvB like protein.

RuvB1 from *L. donovani* is 458 amino acids long protein and its theoretical pI is 5.76 and molecular weight is ~50 kDa. While RuvB2 from *L. donovani* is 483 amino acids long protein and its calculated pI is 5.29 and molecular weight is ~54 kDa.

We further analyzed the LdRuvBs sequences with the RuvB like proteins of *Plasmodium falciparum*, *Plasmodium vivax*, yeast, and human using multiple sequence alignment and result shows that both the RuvB like proteins contain Walker motif A and Walker motif B (**Fig. 3 and 4**). Both these motifs are well known for the ATP binding and for the ATPase activity. Through mutational study of both these motifs it has been discovered that these are essential for the characteristic ATPase activity of RuvB like protein in different organisms including yeast and human.

LdRuvB1 was aligned with human pontin and yeast Rvb1 and all the characteristic motifs of RuvB like protein are boxed in the green color (**Fig. 3**). Thus, from this study it is clear that LdRuvB1 protein contains Walker motif A, Walker motif B, sensor-I, arginine finger motif, and sensor-II motif. Similarly LdRuvB2 was aligned with reptin of human and yeast Rvb2, and the result shows that LdRuvB2 also contains all the characteristic motifs shown in **Figure 4**. Interestingly, both the proteins also contain characteristic DNA binding domain (**Fig. 2**).

The bioinformatics based analysis was performed to obtain the structural insight of all these RuvB like proteins of R2TP complex. For structural modeling the sequences of both the *L. donovani* RuvBs were used for the prediction of 3-dimensional structure of these proteins using Swissmodel homology-modeling

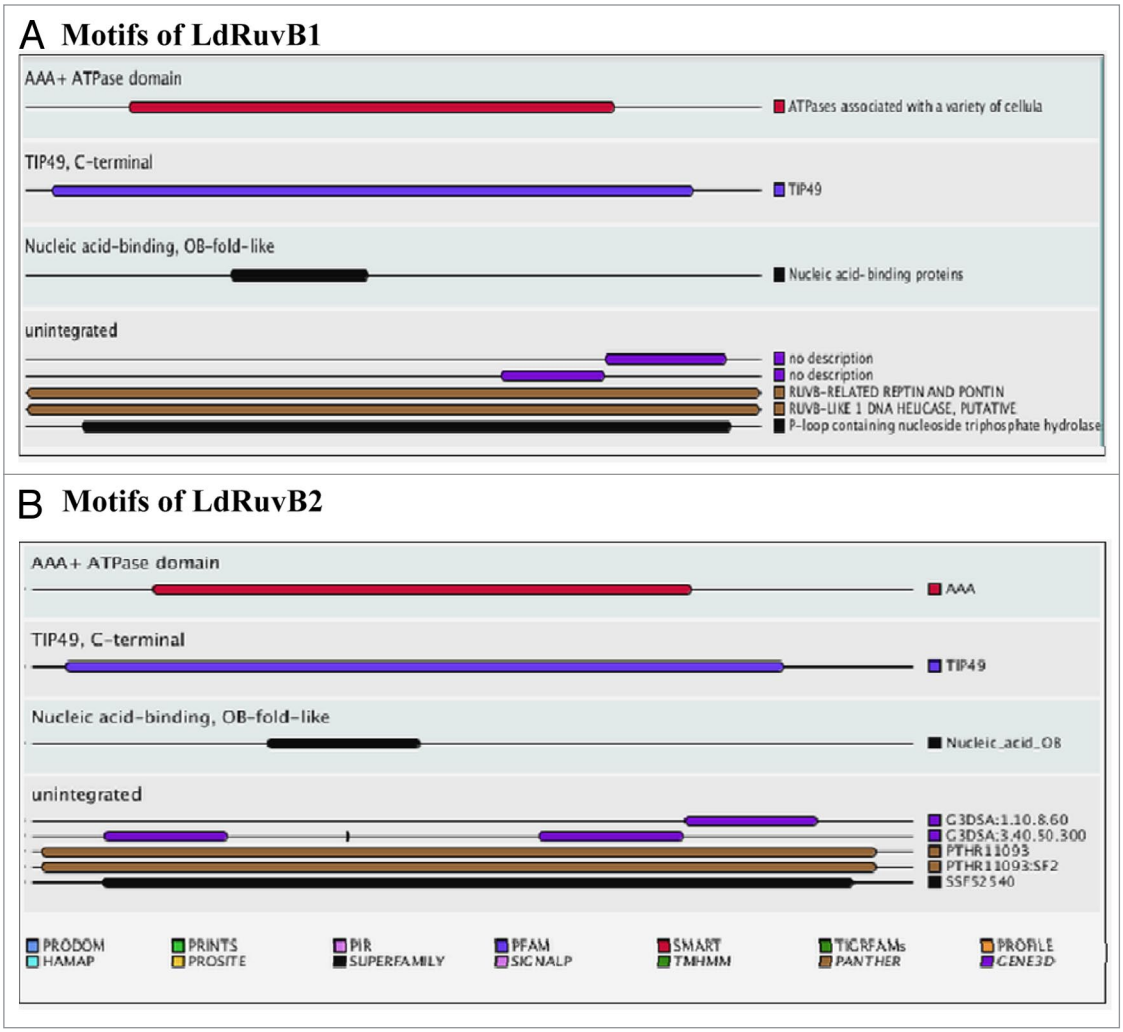

Figure 2. In-silico prediction of conserved motifs of RuvB from *L. donovani* using InterProScan. (**A**) Predicted motifs model of *L. donovani* RuvB1 was prepared, which shows that LdRuvB1 contains characteristic AAA+ domain and belongs to pontin (RuvB like1 helicase). (**B**) Conserved motifs model of *L. donovani* RuvB2 was prepared, which shows that Ld RuvB2 contains characteristic AAA+ domain and belongs to Reptin (RuvB like2 helicase).

server (http://swissmodel.expasy.org/).[17] LdRuvB1 primary sequence residues 12 to 451 showed ~64% identities to human RuvB-like 1 helicase. Structural modeling of the *L. donovani* RuvB1 (http://swissmodel.expasy.org) provided a basic tool to compare with human RuvB1 structure. The ribbon diagram of the template is shown in **Figure 5A** (i) and the predicted structure of LdRuvB1 is shown in **Figure 5A** (ii). The ribbon diagram of the template bound with ADP (known crystal structure of this homolog, PDB number 2C9Oa at http://www.rcsb.org) and the predicted structures of LdRuvB1 were superimposed, it is clear that these structures superimpose considerably and seems that both the proteins have similar ADP binding site [**Fig. 5A** (iii)]. LdRuvB2 primary sequence residues 21–454 showed ~51% identity to a different human RuvB-like helicase from *H. sapiens.* Therefore the structural modeling of the LdRuvB2 was done using the known crystal structure of this homolog as the template (PDB number: 2XSZD at http://www.rcsb.org). The ribbon diagram of the template [**Fig. 5B** (i)] and the predicted structure of LdRuvB2 [**Fig. 5B** (ii)] were superimposed [**Fig. 5B** (iii)] and molecular graphic images were produced using the UCSF Chimera package (http://www.cgl.ucsf.edu/chimera) from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081).[18] These results show that the structure of LdRuvB1 is almost similar to the human RuvB1 while LdRuvB2 has entirely different structure and shares similarity with Reptin (human RuvB2). Thus, it is highly expected that LdRuvB1 and LdRuvB2 enzymatic activities are similar to the yeast RuvB1 and RuvB2 like proteins, which are essential for the viability and are involved in multiple cellular processes.

Further phylogenetic analysis was performed for RuvB like proteins of *L. donovani* with human, yeast, Trypanosoma, Xenopus, *P. falciparum*, *Plasmodium vivax,* and *Plasmodium knowlesi*. Phylogenetic analysis further shows that LdBPK_343280.1 is closer to the Pontin/Rvb1, while LdBPK_342440.1 is closer to the Reptin/Rvb2 (**Fig. 5C**). Thus, these results further confirm the identification of RuvB like1 and RuvB like 2 proteins in the *L. donovani* and we named

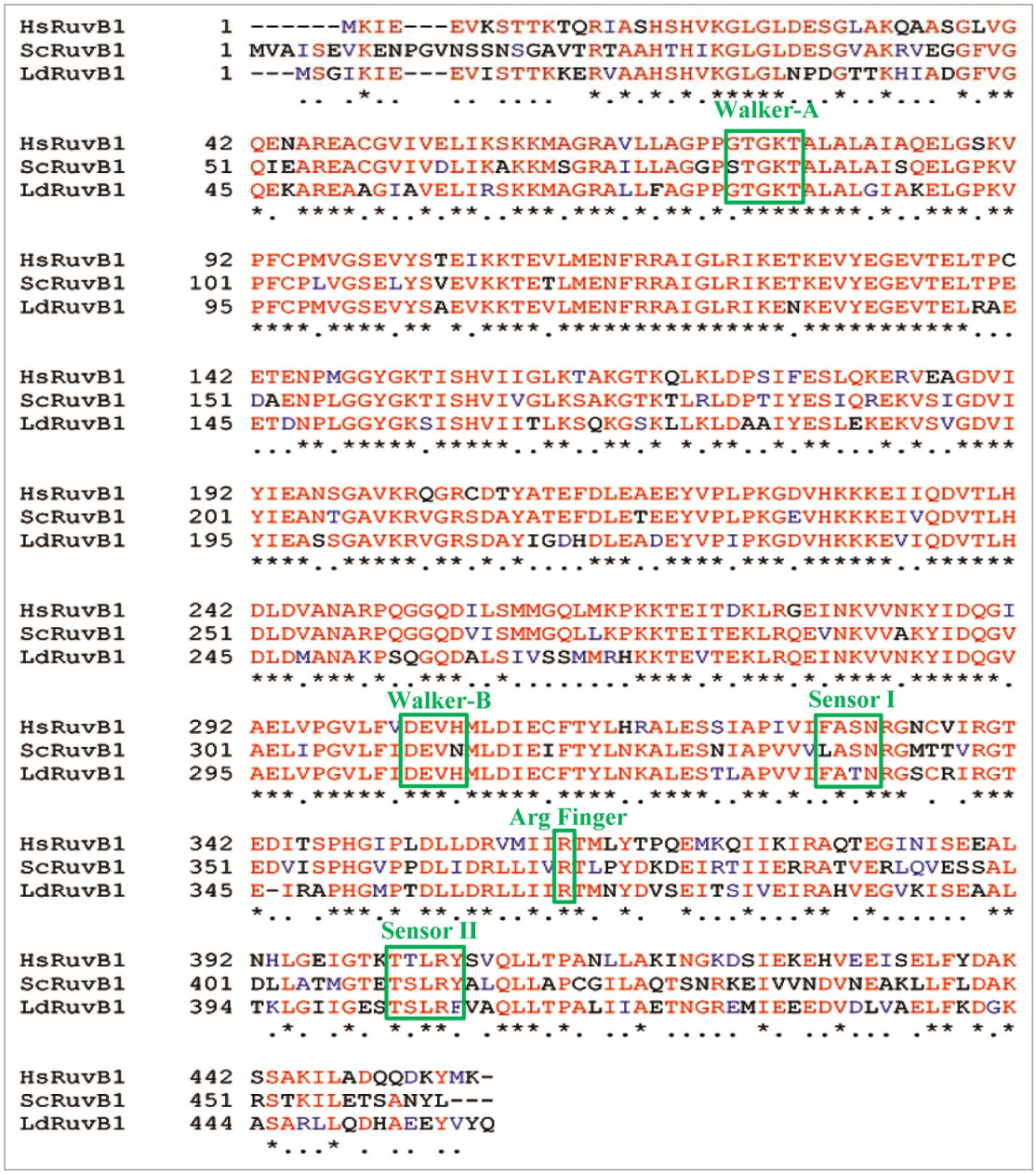

Figure 3. Conserved motifs of *L. donovani* RuvB1 and comparison with yeast and human RuvB1 characteristic motifs. Multiple sequence alignment of LdRuvB1 with yeast and human RuvB1 using ClustalW at default parameters. The amino acid sequence of various conserved motifs like Walker A, Walker B, sensor I, arginine finger, and sensor-II motifs are boxed in green color.

these proteins LdRuvB1 and LdRuvB2, accordingly. In R2TP complex, RuvB1/ RuvB 2 has been found to tightly interact with another component of the same complex that is Pih1 originally discovered in yeast and later in humans.[1]

For the identification of Pih1 in *L. donovani,* we applied the same strategy as for the identification of LdRuvB1 and LdRuvB2. Pih1 sequence of yeast was used for BLAST-P search in the *L. donovani* genome and the results showed that it contains one protein quite homologous to the query sequence with high score. The Genedb number of LdPih1 is Ldbpk_354400.1, which is 635 amino acids long and its calculated pI is 5.55 and molecular weight is ~70 kDa. To further confirm this identification, we retrieved the LdPih1 sequence and BLAST-P was performed in the human genome, which shows that LdPih1 is similar to the human Pih1 and shares 31% identity. We further analyzed the sequence of LdPih1 and PfPih1with InterProScan[19] and the results show that PIH domain is present in both the sequences (**Fig. 6A and B**). The phylogenetic analysis of Pih1 amino acid sequences of human, yeast and *L. donovani,* etc., showed that LdPih1 is closer to the human PIH1 (**Fig. 6C**). In human and yeast, Pih1 acts as an adopter protein, which interacts with RuvB1/RuvB2 through N-terminal, while through C-terminal

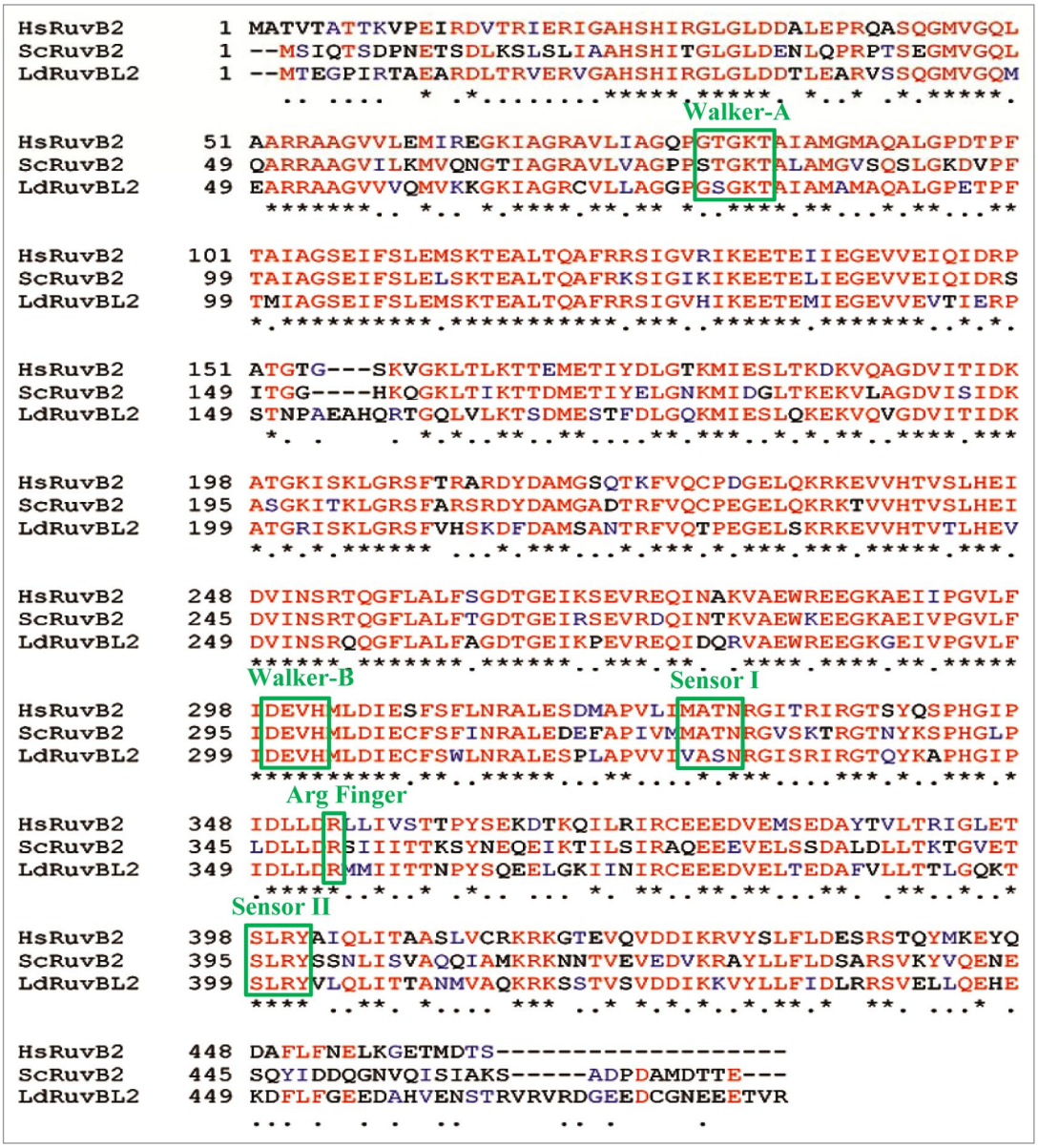

Figure 4. Conserved motifs of *L. donovani* RuvB2 and comparison with yeast and human RuvB2 characteristic motifs. Multiple sequence alignment of LdRuvB2 with yeast and human RuvB2 using ClustalW at default parameters. The amino acid sequence of various conserved motifs like Walker A, Walker B, sensor I, arginine finger and sensor-II motifs are boxed in green color.

it interacts tightly with Tah1, another protein of the same complex. Tah1 contains characteristic TPR motifs, which are known to mediate the interaction with Hsp90 to form R2TP-Hsp90 complex.[20] In *L. donovani,* BLAST-P analysis of yeast/human Tah1 amino acid sequence led to the identification of Tah1 like protein, which is comparable to the human RPAP3 (Tah1 homolog), thus we named this protein as LdRPAP3 and its GeneDB number is Ldbpk_081020.1. Yeast Tah1 protein has only 111 amino acid and its homolog in human (RPAP3) has 665 amino acid while LdRPAP3 contains 546 amino acid. The sequence alignment of LdRPAP3 with human RPAP3 showed only 28% identity. Further amino acid sequence of LdTah1 like protein was studied in silico with InterProScan and the results show the characteristic feature like tricopeptide repeats motif (TPR motifs), which seems quite similar to the human RPAP3 (**Fig. 7A**). These results clearly show that *L. donovani* Tah1 and PfTah1 homologs are close to the human RPAP3, especially in terms of the number of amino acid and the arrangement of TPR repeat (**Fig. 7A and B**). This analysis was further supported by the phylogenetic analysis, which shows that *L. donovani* Tah1 is closer to the human RPAP3 as compared with the yeast Tah1 (**Fig. 7C**).

Identification of the R2TP complex in human malaria parasite *Plasmodium falciparum*

Two main components of the R2TP complex, such as RuvB1/RuvB2, have been already identified and characterized

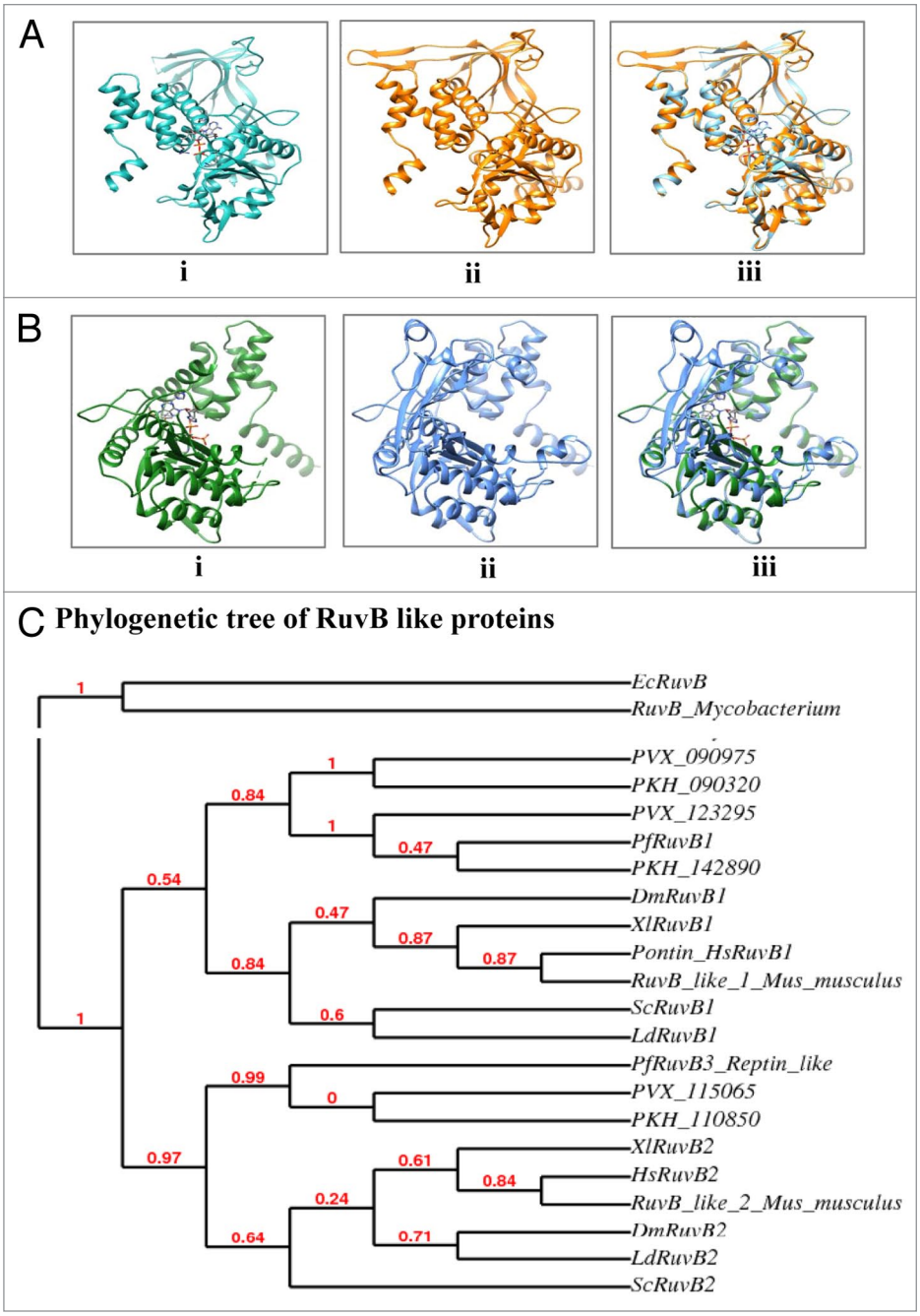

Figure 5. Structure modeling. The modeling of LdRuvB1 and LdRuvB2 was done using amino acid sequences at Swissmodel server. The molecular graphic images were produced using the UCSF Chimera package from the resource for Biocomputing, Visualization, and Informatics (http://www.cgl.ucsf.edu/chimera) at the University of California, San Francisco (supported by NIH P41 RR-01081). (**A**) (i) Template for LdRuvB1; (ii) LdRuvB1; (iii) superimposed image of template and LdRuvB1. (**B**) (i) Template for LdRuvB2; (ii) LdRuvB2; (iii) superimposed image of template and LdRuvB2. (**C**) Phylogenetic analysis of LdRuvB proteins with other RuvB proteins. The phylogenetic analysis of LdRuvB like proteins with *E. coli*, yeast and human RuvBs was carried by using Phylogeny.fr. with default parameters.

recently.[21-23] It is well known that 2 RuvB like proteins (RuvBL1/RuvBl2 or Rvb1/Rvb2 or Pontin/Reptin) are present in yeast to human while the identification of 3 RuvB like protein in *P. falciparum*[21] opened the new avenue to explore the role of these proteins in malaria parasite. It is interesting that 3 RuvB like protein have also been identified in many parasites like *P. vivax*, *P. knowlesi*, *Trypanosoma cruzi,* and *Schistosoma mansoni*.[22] Hence, it seems that mode of regulation of RuvBs in these human parasites shares similar mechanism to control the RuvB like proteins led cellular processes.

The phylogenetic analysis has revealed that PfRuvB1 and PfRuvB2 are closer to human/yeast RuvB like1 (Pontin/Rvb1), while PfRuvB3 seems closer to the human/yeast RuvB like2 protein (Reptin/Rvb2). Recently the detailed characterization of PfRuvB1 confirmed its biochemical role as it contains both ATPase as well as DNA helicase activity. Interestingly, PfRuvB1 also showed some DNA-independent ATPase activity, thus it was speculated that in vivo it can serve as DNA independent ATPase.[7] However, the presence of ss-circular/linear DNA showed enhancement in the ATPase activity of PfRuvB1. Furthermore, the enhancement of ATPase coupled helicase activity in PfRuvB1 indicates its involvement in the nuclear functions like chromatin remodeling and DNA damage repair etc. PfRuvB3, which is phylogenetically closer to human/yeast RuvB2, shows that it contains DNA dependent ATPase activity, but lacked DNA helicase activity from recombinant protein, but endogenous protein showed some helicase activity and Walker motif A is essential for the ATPase activity.[7]

For the identification of Pih1 homolog in malaria parasite, initially we retrieved the yeast and human Pih1 sequences and BLAST-P analysis was performed in the *P. falciparum* genome and results show that it contains one protein with high score and its Plasmodb number is Pf3D7_1235000. This gene was further analyzed by the BLAST-P in the human genome, which shows that PfPih1 is similar to the human PIH1D1 and shares ~27% identity and contains characteristic PIH1 domain. We further analyzed the amino acid sequence of PfPih1 with human PIH1D and yeast Pih1, which showed that PfPih1 aligned considerably with human and yeast Pih1 and it contains characteristic domain of Pih1 (**Fig. 6B**). The phylogenetic analysis of Pih1 amino acid sequences of human,

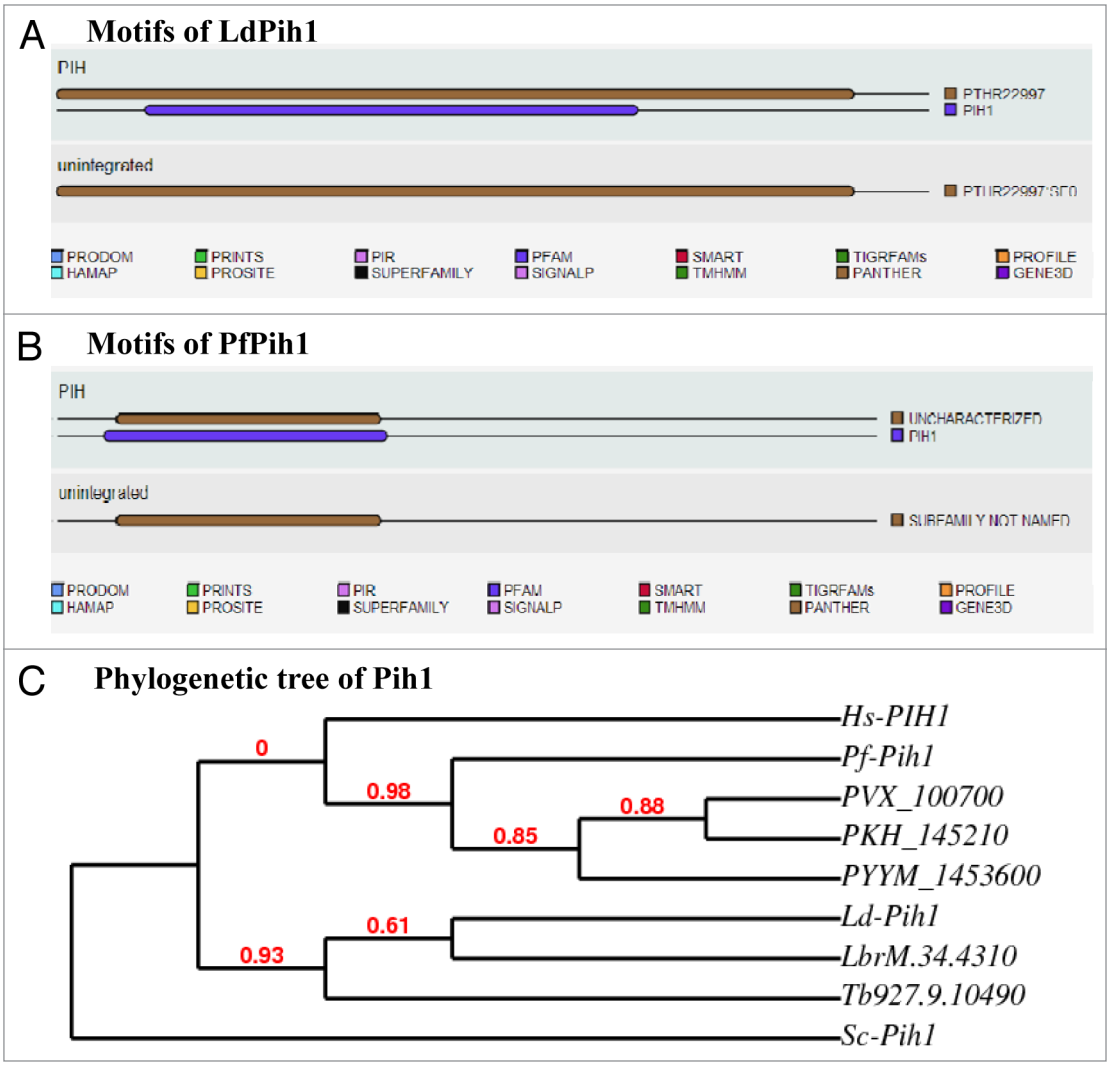

Figure 6. In-silico generated motifs model of Pih1 from *L. donovani*. (**A**) Predicted motifs model of LdPih1 was prepared with InterProScan which shows that LdPih1 contains characteristic PIH domain. (**B**) Predicted motifs model of PfPih1 was prepared with InterProScan, which shows characteristic PIH domain. (**C**) Phylogenetic analysis of LdPih1 *and* PfPih1 proteins with other Pih1 proteins was carried by using Phylogeny.fr.

yeast, *P. falciparum* and *L. donovani* etc. showed that PfPih1 is also closer to the human PIH1 similar to the LdPih1 (**Fig. 6C**).

Similarly, we also performed the bioinformatics based analysis to identify Tah1 protein in *P. falciparum* genome. Using the approach described above, yeast Tah1 sequence was used as query and the results show that Plasmodb contains at least 2 proteins (Pf3D7–1434300 and Pf3D7_0213500) with TPR repeats. Further, these protein sequences were extensively analyzed in silico with the Pfam, ScanProsite, SMART, PANTHER, etc., to identify the characteristic motifs and the results showed that Pf-3D7–1434300 possess similar pattern of the TPR motifs as in the yeast and human Tah1. All these results showed that PfTah1 protein seems similar to the human RPAP3 (Tah1), thus we named this protein as PfRPAP3. Yeast Tah1 protein has only 111 amino acids, RPAP3 has 665 amino acids, while PfRPAP3 has 564 amino acids. The sequence alignment showed that PfRPAP3 is close to the human RPAP3 especially in terms of the number of amino acid and number of TPR repeat (**Fig. 7B**) similar to the *L. donovani* RPAP3 homolog. For structural modeling the sequences of LdTah1 and PfTah1 were submitted to the Swissmodel homology modeling server (http:swissmodel.expasy.org). A number of models were obtained for each sequence, but the models that were built using yeast co-chaperone Sti1/Hop as template were studied in detail. LdTah1 primary sequence residues 226 to 467 showed ~38% identity to the template yeast co-chaperone Sti1/Hop.[24] The modeling of the template is shown in **Figure 8A** and of LdTah1 using this template is shown in **Figure 8B.** When the modeled structure of LdTah1 and the template were superimposed, it is clear that these structures superimpose partially (**Fig. 8C**). PfTah1 primary sequence residues 242 to 491 showed ~37% identity to the template yeast co-chaperone Sti1/Hop.[24] The modeling of PfTah1 using the same template is shown in **Figure 8D**. When the modeled structure of PfTah1 and the template were superimposed, it is clear that these structures superimpose partially (**Fig. 8E**). Molecular graphic images

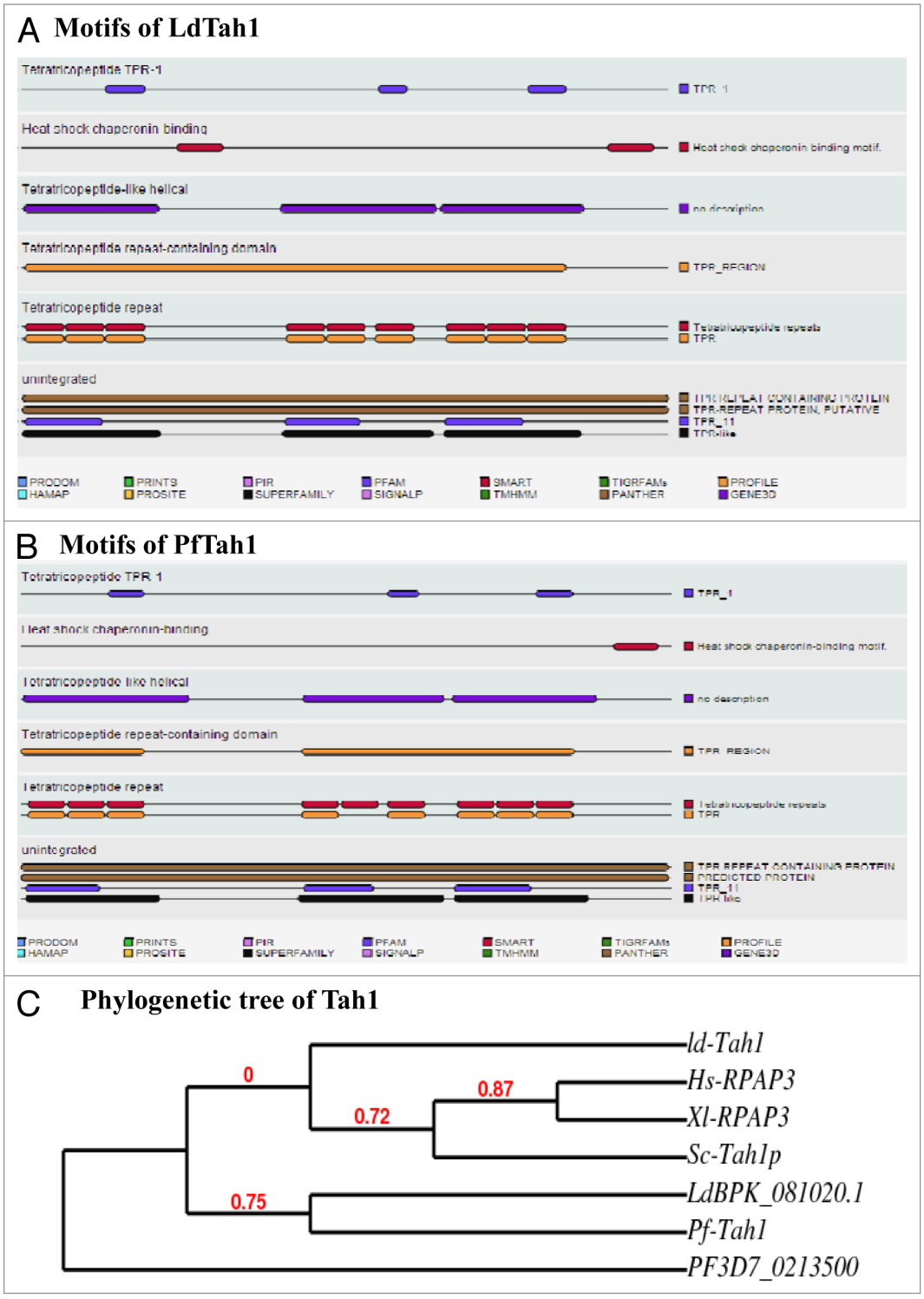

Figure 7. Conserved motifs of Tah1 from *L. donovani and P. falciparum*. (**A**) Predicted motifs model of LdTah1 was prepared with InterProScan which shows that LdTah1 contain characteristic TPR domain. (**B**) Predicted motifs model of PfTah1 was prepared with InterProScan which shows characteristic TPR domain. (**C**) Phylogenetic analysis of LdTah1 *and* PfTah1 proteins with other Tah1 including yeast and human Tah1 proteins was carried by using Phylogeny.fr.

were produced using the UCSF Chimera package (http://www.cgl.ucsf.edu/chimera) from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR-01081).

Considering all the results of these investigations it seems that both parasites have similar kind of R2TP complex, which is composed of RuvB1/ RuvB2, Pih1, and RPAP3 proteins as has been discovered in the yeast and in human.

Concluding Remarks

R2TP complex is a recently discovered important multiprotein complex involved in diverse cellular process like snoRNP biogenesis, PIKK signaling, RNA polymeraseII assembly and in the apoptosis. Pih1 act as adopter protein which tightly interacts with Rvb1-Rvb2 and with the Tah1 to form R2TP macromolecular complex.[11] Tah1 of the R2TP complex further interacts with the Hsp90 to form R2TP-Hsp90 complex that has been found playing role in many cellular process.[25] During this study, the genome wide in-silico screening of the *L. donovani* and *P. falciparum* led to the identification of RuvB like1, RuvB like2, Pih1, and Tah1 genes. The in-silico characterization of conserved motifs helped further to speculate that this complex is also important for these parasites as in the yeast. Detailed analysis of crucial components of R2TP complex, LdRuvB like 1 and Ld-RuvB like 2, revealed the characteristic motifs like DNA binding motif and ATPase motifs. The phylogenetic analysis shows that PfRuvB1, PfRuvB2, and LdRuvB1 are closer to the yeast/human RuvB1, while PfRuvB3 and LdRuvB2 are closer to the yeast/human RuvB2 (reptin). Recently, in many studies it has been reported that RuvB like proteins are overexpressed in many cancers.[8] Later, it was found that the ATPase activity is crucial for the cancer cell proliferation and has been proposed as suitable drug target.[26] Similarly, one of the *P. falciparum* RuvB like protein (PfRuvB3) has been found specific to the stage where rapid nuclear division led multiplication of parasite occurs.[23] Considering all these studies it seems that exploring the role of the parasite R2TP complex may reveal some critical roles in rapid multiplication of the parasite in human host.

Materials and Methods

All the sequences of the components of the R2TP complex from human and yeast were retrieved from their genome database. The downloaded sequences were used for BLAST-P analysis in human genome and yeast genome. All the corresponding sequences of *Leishmania donovani* and *Plasmodium* used in this analysis were retrieved from GeneDB (www.genedb.org) and PlasmoDB (plasmodb.org), respectively. The retrieved corresponding sequences were in-silico studied and various domains were manually assigned and confirmed by using Pfam, Prosite, SMART, PANTHER, etc., integrated software, InterProScan.[19] Multiple-sequence alignment was done by using Mac Vector, as well as ClustalW, while phylogenetic tree analysis was done by using Phylogeny.fr. The ribbon diagram of the template and the predicted structure of LdRuvB1 and LdRuvB2 were superimposed. Molecular graphic images were produced using the UCSF Chimera package (http://www.cgl.ucsf.edu/chimera) from the Resource for Biocomputing, Visualization, and Informatics (supported by NIH P41 RR-01081).[18]

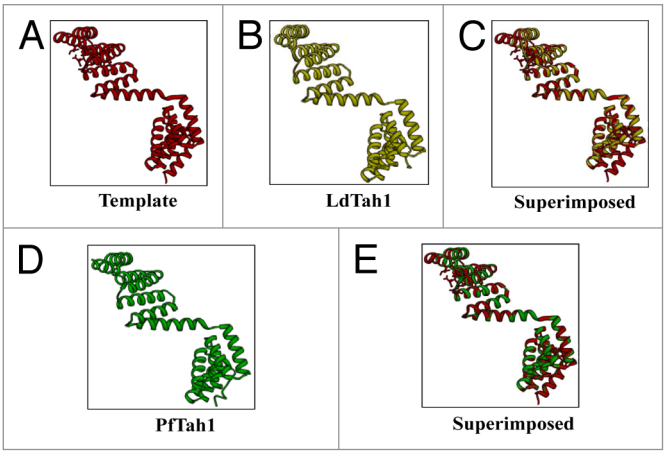

Figure 8. Structure modeling of Tah1 protein. The modeling of LdTah1 and PfTah1 was done using amino acid sequences at Swissmodel server. (**A**) Template, (**B**) LdTah1, (**C**) Superimposed image of template and LdTah1, (**D**) PfTah1, (**E**) Superimposed image of template and PfTah1. The molecular graphic images were produced using the UCSF Chimera package from the resource for Biocomputing, Visualization, and Informatics (http://www.cgl.ucsf.edu/chimera) at the University of California, San Francisco (supported by NIH P41 RR-01081).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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