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Review

Molecular Functions and Potential Utilization of Zinc Finger Proteins in Protozoan Parasites

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ABSTRACT

Zinc finger proteins (ZFP) are metalloproteins whose zinc atom interacts with side chains of specific histidines and cysteines of the zinc finger motif, generating functional three dimensional structures. A good number of these proteins from different species are similar while others are divergent in structure and function. Existing literature on the structural features, functions of these proteins and their potential to be targeted for drug development or used as molecular tools in parasitic protozoa was explored. Evidence from reviewed articles revealed that, just like in other eukaryotes, these proteins function in various parasite cellular processes such as: transcription, RNA editing, mRNA processing & export and, mRNA turn-over among others. Some of them had the same structural features and function as their homologues in other eukaryotes including their hosts. However, there were others which had divergent structure and function. Some studies on drug development aiming at replacing the zinc atom reported positive results in some parasites such as *Giardia lamblia, Trichomonas vaginalis* and *Leishmania donovani* among others. However, though this is promising, the off- target challenge that can cause side effects, must be solved before wide application can be achieved. Successful engineering of these proteins and their use in genome editing was also reported in *Plasmodium falciparum*. Since zinc finger nucleases are a powerful research and medical tool, repurposing of the divergent ZFPs can be useful in the drug target and therapy discovery. For this to succeed, more knowledge on the specific functions of more parasite ZFPs and how their functions are regulated is needed.

Keywords: ZFP, parasite, mRNA.

1 Introduction

Zinc finger proteins are a class of conserved proteins whose structure is stabilized by zinc atom(s) (Hudson et al., 2004), (Farina et al., 2011). In most cases the zinc atom is coordinated by a certain number of specific amino acids, usually cysteins (C) and histidine (H). The resultant structure is known as a zinc finger domain/motif. Variations in the number of cysteins and histidines that coordinate a zinc atom and; the order in which they are arranged define a given class of zinc fingers (Krishna et al., 2003), (Kamaliyan & Clarke, 2024). There are several different classes of zinc fingers which includes; CCHH, CCHC, CCCH, C3HC4 (RING finger) HCCC and Zn2/Cys6 among others (Farina et al., 2011). Each of these zinc fingers either interact with proteins, DNA or RNA thereby mediating the function of these proteins (Krishna et al., 2003), (Cassandri et al., 2017), (Ngwa et al., 2021), (X. Li et al., 2022), (Gosztyla et al., 2024). As a result, proteins with a certain type of zinc finger have conserved roles. For example proteins with CCCH type zinc fingers interact with RNA and are mostly involved in RNA metabolism (Kramer et al., 2010). Their conserved nature and specificity is utilized in functional genomics while functional divergence is used in the discovery of drug targets for various viral pathogens and even cancer among others (Cassandri et al., 2017), (Abbehausen, 2019), (Hajikhezri et al., 2020), (Momin et al., 2023), (Jen & Wang, 2016), (S. Liu et al., 2023), (Zhao et al., 2023). Similarly, this is helpful in functional analyses and identification of unique cellular processes that can be targeted for drug development in parasitic protozoa. In addition, due to their specificity, attempts are being made to engineer specific zinc finger proteins that can target certain parasite

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cellular processes (Nain et al., 2010), (Nakata et al., 2012), (Nomura & Sugiura, 2007), (Papworth et al., 2006), (Straimer et al., 2012), (Moraes Barros et al., 2015).

This paper reviews published literature on the functions of this important class of proteins and highlights advances made in their use in the study and management of infectious protozoan parasites. Structural features and functions of the analyzed ZFPs from various parasites are highlighted and discussed. This is then followed by the exploration and discussion of advances made in targeting these proteins for drug development and their utilization as molecular tools. Milestones and suggestions on future perspectives are presented at the end of this article. Utilization of information presented here will hopefully contribute to advancement of research on these proteins and eventually provide new avenues for drug development against these parasites.

2 Transcription

Several proteins involved in transcription, including transcription factors of different organisms, have zinc finger motifs which mediate their functions (Brown, 2005). Owing to the conserved nature of these proteins, the same was also reported in parasites. In trypanosomes for example, RBP12 subunit of RNA PolII has $CX_2CX_{(10\text{-}15)}CX_2C$ zinc finger motif (Das et al., 2006), while TFIIB that interacts with RNA PolII RBP1 and is involved in transcription of the spliced leader (SL) RNA has a $CX_2CX_{16}CX_2C$, zinc ribbon (Schimanski et al., 2006). Similarly the *Schistosoma mansoni* SmZF1, has three CCHH zinc finger motifs (Cys $X_{2\text{-}4}$- Cys-X_3- Phe- X_5- Lue- X_2- His-$X_{3\text{-}5}$- His (Drummond et al., 2009). It localizes in the nucleus, binds to both DNA and RNA oligos *in vivo* and stimulate transcription in COS cells and is therefore likely to be a transcriptional factor (Eleutério de Souza et al., 2001), (Calzavara-Silva et al., 2004); (Drummond et al., 2009). On the other hand the *Trichomonas vaginalis* TvZFN1 which has eight C_2H_2 zinc finger motifs was shown to bind DNA and is likely to play a role in the regulation of transcription of some genes in this parasite (Villalpando et al., 2017); (Torres-Romero et al., 2020). However, other than the reported general function, little is known about the exact role played by each of these zinc finger motifs.

3 mRNA processing

mRNA processing is done by proteins which have both the ability to bind mRNA and cleave it. A protein known as cleavage polyadenylation specific factor (30kDa) CPSF30 plays an important role. Trypanosomes have homologs of this protein. The TbCPSF30, though small in size, is an essential protein characterized with seven zinc finger motifs. Five of these fingers are of the CCCH type while two are CCHC type. The two CCHC knuckles are located toward the C terminal. Orthologs of TbCPSF30 from other eukaryotes have a comparable number of zinc fingers which are arranged in a similar manner (Bai & Tolias, 1996), (Barabino et al., 2000), (Delaney et al., 2006). *In vivo* studies using tubulin polycistronic RNA transcript revealed that this protein is required for pre-mRNA processing, similar to its orthologues in other species (Addepalli & Hunt, 2007). This function was shown in some species to depend on the CCCH motifs. For example some of the CCCH motifs were reported to be essential for RNA or protein interaction while others have endonuclease activity (Bai & Tolias, 1996); (Barabino et al., 2000), (Addepalli & Hunt, 2007), (Addepalli & Hunt, 2008). While the functional roles of the CCCH and CCHC motifs of this protein in other species are known, that of Trypanosomes and other parasites are not known. It is therefore worthy analyzing whether the CCCH motifs of parasite CPSF30 have similar or diverged activities. The other protein involved in mRNA processing is Trypanosome U2AF35 (U2 auxiliary factor 35) that plays a role in splicing (Vazquez et al., 2009), (Gupta et al., 2013). Both TbU2AF35 and Tc2AF35 have two CCCH motifs (Vazquez et al., 2009). Similarly, TcFIP-1 (Factor Interacting with PAP (Poly(A) Polymerase) -1) like, which also contains a CCCH zinc finger not found in other homologues, plays a role in the poly(A) tail processing of trypanosome mRNAs. It was also reported to interact with TcCPSF30 (Bercovich et al., 2009).

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4 RNA editing

RNA editing, quite commonly occurs on trypanosome mitochondrial mRNAs. Editing is done by a complex known as editosome and involves the removal or addition of nucleotides (mostly uridine) to an existing RNA molecule. There are three sub-complexes made up of about 12 common proteins. Of these proteins, KREPA2 (kinetoplastid RNA editing protein A2)/TbMP63 and KREPA3/TbMP42 have two C2H2 zinc fingers towards their N-terminus while KREPA1/MP81 has one such zinc finger and another which is zinc finger like. The Leishmania homologs of these proteins are LC-4 (Leishmania complex- 4), LC-7b and LC-1 respectively. The *Trypanosoma brucei* KREPA1 and KREPA3 are essential for both Blood stream and Procyclic parasite RNA editing (Drozdz et al., 2002), (Guo et al., 2008). In addition to the zinc fingers, these three proteins have an OB (oligonucleotide/oligosaccharide binding)- fold towards the C-terminus 2 (Kang et al., 2004), (Panigrahi et al., 2001). The trypanosome OB fold mediate protein-protein interactions which links either of the first two sub-complexes (that catalyzes uridityl removal and addition) with the ligase sub-complex (Panigrahi et al., 2001), (Schnaufer et al., 2010). KREPA3 plays a central role in this structural organization by linking sub-complexes in the editosome (Guo et al., 2008). The zinc fingers of this protein were found to be essential for *in vivo* editing though, the particular role was not identified. In contrast, the zinc finger domains of *Leishmania tarentolae* LC-4, a homolog of TbKREPA2/TbMP63 are the ones that mediate protein-protein interactions thereby stabilizing the complex. Mutational analyses of LC-4 revealed that zinc finger-1 holds the complex together, more than zinc finger- 2 (Kang et al., 2004). The cause of this disparity in the way these protein domains function in these related species is not clear.

Three trypanosome proteins KREN1 (kinetoplastid RNA editing nuclease 1)/KREPB1/MP90, KREN2/KREPB3/MP61 and KREN3/KREPB2/MP67 also have a C2H2 zinc finger domain, an RNase III domain and a dsRBM domain. All these nucleases are essential for mRNA editing and parasite survival (Worthey et al., 2003), (Carnes et al., 2005), (Trotter et al., 2005), (Carnes et al., 2008), (Carnes et al., 2012), (Lerch et al., 2012), (Carnes et al., 2023). While the role of the nuclease domain is mRNA cleavage and editing is clear, more data is required to affirm and elucidate the proposition that the zinc finger domains control interactions between RNA to be edited and gRNA (guide RNA) (Carnes et al., 2022).

Four other proteins KREPB4/MP46, KREPB5/MP44, KREPB6/MP49, KREPB7/MP47 and KREPB8/41 also have a U1- like, C2H2 zinc finger motif at their N terminus (Worthey et al., 2003), (Carnes et al., 2012), (Lerch et al., 2012). Other than KREPB4/MP46 and KREPB5/MP44 (Wang et al., 2003), (Babbarwal et al., 2007), (Carnes et al., 2012) which have an additional RNase III domain overlapping a PUF (Pumilio and FBF) domain, the rest have only the zinc finger domain (Schnaufer et al., 2010), (Carnes et al., 2012b). Mutational analyses of the RNase domain found them none catalytic though the two proteins form a heterodimeric complex with the catalytically active editing endonucleases. Similar analyses on the zinc finger residues revealed that this domain was essential for the functioning of KREPB4 in maintaining editosome integrity (Carnes et al., 2012b). It is however not clear what exactly this motif does. In addition the role of the zinc finger of the remaining four proteins has not been evaluated conclusively.

5 mRNA export

mRNA export in eukaryotes is done by a combined effort of various proteins (such as Mex67 – Messenger RNA EXport Factor of 67 kDa and karyopherins) which directly interact with the mRNA. Homologs of Mex67 are also present in some protists. The trypanomastid Mex67 contain a CCCH zinc finger motif which is lacking in its orthologues in *Saccharomyces cerevisiae* and mammals (Kramer et al., 2010), (Dostalova et al., 2013). RNAi depletion of *T. brucei* Mex67 and mutation of its zinc finger motif led to nuclear accumulation of poly(A^+) mRNA (Schwede et al., 2009), (Dostalova et al., 2013). In addition, this protein, co-localizes with nucleoporins and interacts with TbMtr2 (Mtr2- MRNA TRansporter 2) and a karyopherin TbIMP1 (IMP1- Inner Membrane Protease 1) (Kramer et al., 2010), (Dostalova et al., 2013). Similarly, depletion of TgZFP2 a C_2H_2 zinc finger protein which interacts with mRNA splicing and export proteins, caused poly(A^+) mRNAs to accumulate in the nucleus of *Toxoplasma. gondii*, This was rescued by the

Plasmodium PfZFP2 which is also known to be an mRNA export factor (Gissot et al., 2017), (Ngwa et al., 2021). However, though HuZFN2, the human counterpart and TgZFP2 shares sequence similarity in the zinc finger motif, however it failed to restore TgZFP2 functionality (Gissot et al., 2017) implying divergence in function. These studies clearly demonstrate that these proteins play a role in mRNA export. It's also probable that their orthologues in other related species play a similar role. Structural and functional divergence between parasite and host export machinery can offer a good opportunity for drug targeting.

6 mRNA stability

The first ZFPs to be linked to mRNA stability were the mammalian TTP (tristetraprolin), BRF1 and BRF2 (butyrate response factor 1 and 2 respectively) which have two CCCH ($CX_8CX_5CX_3H$) motifs. Generally, these proteins use these zinc finger motif to select and bind the AREs (AU rich elements) at the 3' UTR (UnTranslated Region) of their target mRNAs (Lai et al., 2000), (Lai et al., 2006), (Brooks & Blackshear, 2013). They also interact with the 5' - 3' xrn1 (exoribonuclease 1) and the exosome thereby targeting bound mRNAs for degradation (Lai et al., 1999), (Carballo et al., 2000), (Stoecklin et al., 2002), (Lykke-Andersen & Wagner, 2005). This discovery and the fact that trypanosomes regulate their gene expression mostly by posttranscriptional mechanisms (Clayton, 2002), attracted many trypanomastid researchers to these class of proteins. An *in silico* analysis of the Trytrip genome revealed that *Trypanosoma brucei* had over 40 genes coding for both conventional and unconventional CCCH ZFPs. *Trypanosoma cruzi* and *Leishmania major* had over 50 genes each. Just like many trypanomastid proteins, many of these proteins have little sequence similarity to their mammalian counterparts (Kramer et al., 2010).

Functional characterization of some of these proteins revealed that TcZFP2 (Mörking et al., 2012), TbZFP3 (P. B. Walrad et al., 2012), TbZC3H11 (Droll et al., 2013), TbZC3H20 (Ling et al., 2011), TcZC3H39 (Alves et al., 2014) and TbZC3H21 (B. Liu et al., 2020) bind or associate with mRNAs. TbZC3H11 has a single $CX_8CX_5CX_3H$ motif, which, together with the sequence preceding it are quite similar to that of TTP (Droll et al., 2013). On the other hand, TbZC3H20 has two $CX_7CX_5CX_3H$ motifs (Ling et al., 2011). Both proteins bind to the 3' UTRs of their target mRNAs in procyclic parasites (Ling et al., 2011), (Droll et al., 2013). TbZC3H11 binds AUU AREs of mRNAs coding for several heatshock proteins (HSP70, HSP83, HSP100, HSP110, HSP20 among others) thereby stabilizing them (Droll et al., 2013). These AREs are quite similar to the classical ones bound by TTP (Lai et al., 2006), (Droll et al., 2013). Stabilization of these mRNAs enhances the expression of heatshock proteins which facilitates procyclic parasite heatshock survival (Droll et al., 2013). On its part, TbZC3H20 binds and stabilizes the mRNAs coding for MCP12 (mitochondrial carrier protein 12) and *TS-like E* mRNA (*trans*- sialidase like E). Though its exact recognition sequence has not been established, however, homopolymeric RNA binding assay revealed a preference for polyG (Ling et al., 2011), (B. Liu et al., 2020). In both cases, the CCCH motif was necessary for efficient RNA binding (Ling et al., 2011). However, what's not quite clear is whether this stabilization results from the prevention of the binding of destabilizing factors i.e. competitive binding/steric hindrance to the binding of the destabilizing factor or whether their role is just divergent. On its part, TcZC3H39 has a CCCH zinc finger motif and a U- box domain. It probably binds to an AAACAA sequence at the 3' UTRs of its target mRNAs in stress granules of starved epimastigote parasites. Though it was speculated to cause translational repression of bound mRNAs which included those encoding nuclear encoded mitochondrial proteins (Alves et al., 2014), more studies to affirm this finding and define the role of the zinc finger motif is required.

TcZFP2 and TbZFP3 have a single $CX_8CX_5CX_3H$ zinc finger motif whose integrity is crucial to their functions in mRNA metabolism (P. Walrad et al., 2009); (P. B. Walrad et al., 2012), (Alves et al., 2014). These proteins have been found to interact with 3' UTR of mRNAs involved in trypanosome differentiation (Mörking et al., 2004); (P. B. Walrad et al., 2012). Though direct mRNA binding assays are yet to be done, however, TbZFP3 was reported to co-precipitate with *GPEET* and *EP1* mRNA of *T. brucei*. Interaction was via loop II and the 16mer regulatory sequences of these mRNAs (Paterou et al., 2006); (P. Walrad et

al., 2009). More interacting mRNAs, most of which are required for parasite differentiation were also reported (P. B. Walrad et al., 2012). Through this interaction, TbZFP3 regulates translation of these mRNAs thereby enabling a switch from GPEET procyclin to EP-1 procyclin (P. Walrad et al., 2009). Similarly, *in vitro* assays revealed that TcZFP2 preferentially bind to A rich sequences at the 3' UTR of target mRNAs. The exact mRNA recognition sequence was however, not conclusively established. Just like TbZFP3, some of the bound mRNAs were upregulated in *T. cruzi* metacyclic trypanomastigote, suggesting a role in parasite differentiation. The other mRNAs code for proteins involved in different cellular processes. Interestingly, TcZFP2 also bound its own mRNA leading to a speculation that it may auto-regulate its expression (Mörking et al., 2004). Though TTP uses a similar mechanism to regulate its expression, however, the role of TcZFP1 in mRNA metabolism is not yet clear. *In vivo* experiments will be required to demonstrate this (Brooks et al., 2004).

The other CCCH ZFPs studied includes TcZFP1, TbZFP1, TbZFP2, TbZC3H12, TbZC3H13 and TbZC3H18 (Hendriks et al., 2001), (Benz et al., 2011), (Ouna et al., 2012). Other than TbZC3H18 which has two $CX_7CX_5CX_3H$ zinc fingers, all the others have a single $CX_8CX_5CX_3H$ motif. In addition to the CCCH motif, TbZFP2 has a WW domain used for protein-protein interaction (Hendriks et al., 2001). TbZFP1 is required for proper kinetoplast repositioning while TbZFP2 causes posterior end cell body extension (Hendriks et al., 2001), (Hendriks & Matthews, 2005). On its part, TbZC3H18 plays a role in the early events of EP procyclin acquisition (Benz et al., 2011). All these events occur during *T. brucei* differentiation from bloodstream form to the procyclic form. Thus these three proteins play essential roles of promoting bloodstream parasite differentiation (Hendriks et al., 2001), (Hendriks & Matthews, 2005), (Benz et al., 2011). Other than these three proteins, the specific functions of the remaining proteins have not yet been identified. In addition interaction of all of these proteins with RNAs has not been established. However, *in vitro* studies showed that TcZFP1 bind polyC rich oligoribonucleotides (Mörking et al., 2004). TbZC3H12 TAP pulled down XNA while TbZC3H18 was pulled down by Tb14-3-3 TAP (Benz et al., 2010), (Benz et al., 2011), (Ouna et al., 2012). Attempts to validate these interactions either failed or were not convincing. Interaction with XRNA is quite interesting as it gives an indication of RNA degradation (C.-H. Li et al., 2006), (Manful et al., 2011). Since TTP interact with similar proteins, perhaps the use of a different experimental strategy, may establish their role in RNA metabolism (Lykke-Andersen & Wagner, 2005), (Brooks & Blackshear, 2013).

7 Nuclease activity

L. donovani LdCSBP (cycling sequence binding protein) has two pairs of divergent CCCH zinc fingers and is likely to play a role in cell cycling. The two fingers of each pair are separated by 9 residues and; read in the opposite direction ($HPCX_6CX_5C$ and $CX_7CX_{5-6}CX_3H$) as if they form a stem loop. It also has an endonuclease Smr domain and two conserved ubiquitin interacting domains (UBA and CUE) (Bhandari & Saha, 2007). *In vitro* assay revealed that LdCSBP has endoribonuclease activity. It uses its CCCH motifs to interact with the target mRNA, LdCyc1 (*L. donovani* cyclin 1) via an octamer (CAUAGAAG) sequence at the 5' UTR (Bhandari et al., 2011). In the *in silico* study done by Kramer et al., another Leishmania protein, LmjF34.1240 was reported to have a $CX_7CX_5CX_3H$ and an exoribonuclease domain (Kramer et al., 2010). The predicted function of this protein has, however, not been confirmed by laboratory experiments.

8 Regulation of the activities of CCCH ZFP proteins

Given the novel roles played by various CCCH ZFPs, attempts to understand mechanisms by which their activities are regulated were made. TTP and BRF 1 were found to undergo phosphorylation and their activities are regulated via this modification (Tchen et al., 2004), (Schmidlin et al., 2004). Interaction with protein 14.3.3 and ubiquitination also play a role in this regulation (Brook et al., 2006), (Sun et al., 2007). To this end several parasite CCCH ZFPs were analyzed. *L. donovani* CCCH protein LdCSBP, was found to be ubiquitinated and its activities are regulated via this modification (Bhandari et al., 2011). Similarly, TbZC3H11, TbZC3H12, TbZC3H13, TbZC3H17, TbZC3H18, TbZC3H27 and TbZC3H44 were shown

to be phosphorylated (Nett et al., 2009), (Benz et al., 2011), (Ouna et al., 2012), (Droll et al., 2013). In addition Tb14-3-3 was reported to interact with phosphorylated proteins including TbZC3H18 (Benz et al., 2010), (Inoue et al., 2010). While it is possible that a regulatory mechanism similar to that used by TTP exists in typanosomes, however, several questions remain unanswered. For example, of what significance is this phosphorylation? Do phosphorylated parasite CCCH ZFPs which control mRNAs stability also interaction with Tb14-3-3? Interaction with Tb14.3.3 is difficult to validate and some of these phophoproteins do not induce a stable traceable phenotype (Benz et al., 2010), (Inoue et al., 2010), (Benz et al., 2011), (Ouna et al., 2012). Proper answers to some of these questions will only be obtained when these challenges have been surmounted.

Three conclusions emerge from these studies; first, trypanosome CCCH ZFPs are involved in different cellular processes. Two, their zinc finger motifs and target mRNA recognition sequences differ. Three and perhaps the most interesting thing, is that the target mRNAs are in most cases stabilized rather than destabilized as is the case with TTP (Lai et al., 1999), (Carballo et al., 2000), (Stoecklin et al., 2002), (Lykke-Andersen & Wagner, 2005). Divergence of parasite CCCH and other types of ZFPs from mammalian ones and variance in the mode of action offer a good opportunity for specific drug and therapeutic design. The number of these proteins studied to date is very small. Functions and cellular processes of many other mammalian and parasite ZFPs are not known (Ngwa et al., 2021). In addition, the mechanism by which their functions are regulated is either lacking or not well elucidated. More research in these areas may give more interesting data and valuable conclusions for drug and therapeutic design.

9 Targeting ZFPs and use of engineered zinc finger proteins for drug and therapeutic designs

Zinc is important for structure stabilization and thus functioning of various zinc metalloproteins. Perturbations of its levels were shown in some studies to be catastrophic to parasite survival. Consequently several zinc containing or zinc chelating drug formulations were tested against Leishmania (Saini et al., 2017), (Aghaei et al., 2024) and *T. vaginalis* (Midlej et al., 2019) among others. Some of these were reported to be effective though more work is required to clarify their mode of action. There are two types of compounds that modulate the functioning of ZFPs. The first class of these compounds act by dislodging the zinc atom of the zinc finger (Farina et al., 2011), (Frézard et al., 2012), (Abbehausen, 2019). This causes a distortion that destabilizes the zinc finger structure. As a consequence, the ZFP is inactivated thereby regulating the affected cellular process. A good example of a substance that functions this way is disulfiram (McDonnell et al., 1997). Disulfiram is used as a drug for many health problems. *In vitro* studies using this drug or its derivatives showed that, it is active against *P. falciparum* (Scheibel et al., 1979), *T. cruzi* (Lane et al., 1996), *G. lamblia* (Nash & Rice, 1998) and *T. vaginalis* (Goodhew & Secor, 2013) among other parasites. Such drugs are likely to suffer from lack of specificity as they will affect the activity of any zinc finger protein. Minimizing side effects may require drugs that act by replacing the zinc atom from the ZFP with other metals. Hopefully, the target protein loses function while most of the others retain their functions. Several studies on other infectious agents based on this approach posted positive results (Cassandri et al., 2017), (Abbehausen, 2019), (Gil-Moles et al., 2020), (Ok et al., 2021), (Dragone et al., 2022). Similar studies on parasitic protozoa may reveal the potential in this approach.

Other types of compounds modulate the ZFPs activity by competitive binding of the active site thereby preventing substrate binding (Farina et al., 2011). This second category of compounds is likely to have high specificity. Nevertheless, published reports on the use of such compounds against parasitic protozoa are scanty. Though most of the studied proteins in these parasites in the preceding sections have other functions other than enzymatic, nonetheless modified compounds such as synthetic oligonucleotides that competitively bind to the zinc finger motif may modulate their functioning, Structural or functional divergence of a good number of the studied protozoan ZFPs from their mammalian homologs offers a good opportunity for drug targeting. It would be interesting to see the outcomes of such studies. On the other hand, rather than targeting the ZFP, a designed ZFP is used to edit the sequence coding for a certain

important protein. For example, a zinc finger nuclease specifically engineered to generate a null mutant by disrupting the DNA sequence coding for the $CD4^+$ T cells CCR5 (C-C motif) receptor 5) receptor was found to confer resistance to HIV-1 infection in mice (Perez et al., 2008), (Schiffer et al., 2012), (L. Li et al., 2013). Further, excision of proviral DNA from cultured human cells genome is now possible (Qu et al., 2013). Though there are inherent challenges in the use of these engineered ZFPs in humans (Schiffer et al., 2012), however, high specificity, effectiveness and improvement in their delivery in model organisms and cultured cells makes their use in gene therapy feasible (McMahon et al., 2011); (Perdigão et al., 2020).

Scientists desperate to combat malaria parasites, proposed a similar approach (Nain et al., 2010). In line with this hypothesis, an *in silico* study which identified potential editing nucleases that can target primordial RBC (red blood cell) receptors for plasmodium merozoites was done (Kajumbula et al., 2012). Concurrent to that study, another group was developing a method for engineering zinc finger nucleases in *P. falciparum*. A nuclease generated using this method was able to edit the DNA sequence of *Pfcrt* gene (Straimer et al., 2012) and *P. vivax Pvdhfr* (Moraes Barros et al., 2015). These initial results laid foundation for *in vitro* and *in vivo* experiments for malaria parasites. Reports on the adoption of similar strategies on other parasites are however, still lacking. Since this is an emerging technology, given advances in zinc finger engineering (Negi et al., 2023) and AI technology in this area (Ichikawa et al., 2023), it will be interesting to see if such strategies can overcome challenges of conventional parasite vaccine development and the ever increasing parasite drug resistance.

Key to the successful design of drugs targeting parasite specific ZFP activities or, the design of ZFPs targeting parasite specific processes, is functional genomics of ZFPs. There are only a couple of reports on functional characteristics of these proteins in parasitic protozoa. More work has been done in trypanosomes compared to other protozoan parasites. This may have been occasioned by a delay in genome sequencing and difficulties in culturing of some of these parasites and; little funding for basic research. However, this is likely to change as the number of sequenced genomes and as reports on the successful drug targeting and use of ZFPs for therapy increases.

10 Conclusion

Zinc finger proteins interact with other proteins, lipids and nucleic acids. In so doing they coordinate and regulate many important cellular processes. They have therefore been engineered for genome editing or targeted for drug development in various species and disease conditions. In this review available literature was explored for similar functions and advances made in their utilization in the management of parasitic protozoa. Recent research revealed that ZFPs play important roles which include transcription, RNA processing and modulation of mRNA stability among others. A good number of these proteins have divergent function from that of other eukaryotes including some of their hosts. This divergence provides an opportunity for drug targeting. Attempts aimed at disrupting the structure of these proteins and thus functionality revealed that some drug formulations based on this approach can be effective, though specificity remains a big challenge. Adoption of artificial intelligence and genetic engineering of ZFPs though still scanty in these parasites, the few available reports showed that they are powerful tools that advanced our knowledge on these proteins. Taken together, the highlighted advance in research provides opportunities that can be explored in the identification of more drug targets or design of specific drugs and genetic therapeutics. Only a small fraction of these proteins has been analyzed and the exact mechanism of action of most of them remains unknown. More work focusing on identifying unique ZFPs; formulating more zinc targeting drugs; design of ZFP based molecular tools and biopharmaceutics may provide solutions to the many medical and economic challenges posed by these parasites.

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11 Declarations

11.1 Study limitation

This review was informed by publicly available articles that I could access. It was not possible to cite all work that has been done on these parasites, though relevant and valuable.

11.2 Acknowledgement

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11.3 Competing Interests

The author declares no conflict of interest.

11.4 Publisher's Note

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