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Morphologic Analysis of the Apicoplast Formation in *Plasmodium falciparum*

Marleen Linzke

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Universidade de São Paulo

Morphologic Analysis of the Apicoplast Formation in *Plasmodium falciparum*
Marleen Linzke

PhD Thesis
University of Groningen, The Netherlands
University of São Paulo, Brazil
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The research described in this thesis was carried out at the Unit for Drug Discovery, Department of Parasitology, Institute of Biomedical Sciences at the University of São Paulo, Brazil and at the Structural Biology Unit, Department of Drug Design, Groningen Research Institute of Pharmacy at the University of Groningen, The Netherlands and was financially supported by an Ubbo Emmius and a FAPESP (project number 2014/23330-9) fellowship, further by the CAPES/Nuffic MALAR-ASP network and Marie Skłodowska-Curie grant Agreement No. 675555, Accelerated Early stage drug discovery (AEGIS).

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Morphologic Analysis of the Apicoplast Formation in *Plasmodium falciparum*

Phd thesis

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and in accordance with
the decision by the College of Deans

and

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on the authority of the
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and in the accordance with
the decision by the College of Deans

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This thesis is dedicated to my mother which always believed in me even when I was not believeing in myself anymore. For all the support and love which made me the person I am today.

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Abstract (Dutch)

LINZKE, M. **Morfologische analyse van de Apicoplast-vorming in *Plasmodium falciparum*** 2019. 109p. Promovendus (Parasitologie) -Instituut van biomedische wetenschappen, Universiteit van São Paulo en Universiteit van Groningen, São Paulo, 2019.

Malaria, veroorzaakt door *Plasmodium spp.*, is jaarlijks met meer dan 400.000 sterfgevallen een van de dodelijkste ziektes wereldwijd. De toename van resistentie tegen huidige medicijnen vormt een grote bedreiging voor de bestrijding en uitroeiing van de ziekte, waardoor nieuwe doelwitten voor geneesmiddelen hard nodig zijn. De apicoplast, een chloroplast-achtig organel van de *Plasmodium* parasiet, is aangetoond essentieel te zijn voor overleving van de parasiet, wat het een interessant te onderzoeken doelwit maakt. Hoe de parasiet dit essentiële organel deelt tijdens de asexuele replicatie is tot nu toe een open vraag geweest. De voorouders van de apicoplast, namelijk de chloroplast en bacteriën, realiseren hun distributie met behulp van eiwitten uit de Min-familie, welke nog niet zijn geïdentificeerd in *Plasmodium spp.* Door intensief BLAST-onderzoek werd een mogelijk ortholoog van een lid van de Min-familie, MinD, geïdentificeerd voor *P. falciparum*. Het ortholoog vertoont de karakteristieke domeinen voor de functie van ATPase die is beschreven voor MinD, en het wordt voorspeld, dat deze gericht is op de apicoplast van de parasiet. Analyse van het recombinante eiwit toont het vermogen aan om het substraat ATP te binden, gevolgd door polymerisatie. Deze effecten zijn afhankelijk en worden versterkt door toevoeging van tweewaardige metalen. Lokalisatiestudies in *P. falciparum* toonden de apicoplast als doelwit aan. Bovendien vertoonde overexpressie van het ortholoog in transgene parasietlijnen een remmend effect op de proliferatie van de parasiet. Met behulp van een referentielijn voor de visualisatie van de apicoplast, zijn technieken voor het visualiseren en analyseren van de apicoplast, door middel van levende cel fluorescentie beeldopnames tijdens het erythrocytische stadium, vastgesteld en geverifieerd voor de analyse van de apicoplast morfologie onder invloed van het mogelijke MinD ortholoog.

Tefwoorden: *Plasmodium falciparum*, apicoplast, live cell imaging, geneesmiddeldoen validatie.

Abstract (English)

LINZKE, M. **Morphologic Analysis of the Apicoplast Formation in *Plasmodium falciparum*** 2019. 109p. Ph.D. (Parasitology) -Institute of Biomedical Sciences, University of São Paulo and University of Groningen, São Paulo, 2019.

Malaria, caused by *Plasmodium spp.*, remains with more than 400.000 deaths per year one of the most severe diseases worldwide. Increasing drug resistance against the available antimalarial drugs poses a great threat in combating and eradication of this disease and new drug targets are greatly needed. The apicoplast, a chloroplast-like organelle of the *Plasmodium* parasite has been shown to be essential for the parasite survival and offers a new drug target to exploit. How the parasite distributes this essential organelle during the asexual replication has been an open question up to this point. The ancestor of the apicoplast, namely the chloroplast and bacteria accomplish their distribution by the aid of proteins from the Min family that has not been identified in *Plasmodium spp.* yet. Through intensive BLAST research, one possible orthologue of one member of the Min family, MinD, was identified for *P. falciparum*. The orthologue displays the characteristic domains for the function of an ATPase described for MinD and is predicted to be targeted to the apicoplast of the parasite. Analysis of the recombinant protein demonstrated its ability to bind to the substrate ATP and to polymerise upon addition of the substrate. This effect is dependent and enhanced by addition of divalent metals. Localisation studies in *P. falciparum* demonstrated the targeting to the apicoplast. Furthermore, overexpression of the orthologue in transgenic parasite lines displayed an inhibitory effect on the proliferation of the parasite. With the help of a reference line for visualisation of the apicoplast, techniques to visualise and analyse the apicoplast by live cell fluorescence imaging during the erythrocytic stage have been established and verified for the analysis of the apicoplast morphology under influence of the possible MinD orthologue.

Keywords: *Plasmodium falciparum*, apicoplast, live cell imaging, Drug target validation.

Resumo

LINZKE, M. **Análise morfológica da formação de apicoplasto em *Plasmodium falciparum*** 2019. 109f. Tese (Doutorado em Parasitologia) - Instituto de Ciências Biomédicas, Universidade de São Paulo e Universidade de Groningen, São Paulo, 2019.

A malária, causada por *Plasmodium spp.*, permanece como uma das doenças infecciosas mais importantes do mundo, sendo responsável por mais de 400,000 mortes por ano. O aumento da resistência aos medicamentos antimaláricos disponíveis representa uma grande ameaça ao combate e a erradicação desta doença e a descoberta de novos alvos são necessários. O apicoplasto dos parasitas *Plasmodium*, uma organela semelhante ao cloroplasto, demonstrou ser essencial para a sobrevivência do parasita e oferece um novo alvo a ser explorado. Como o parasita distribui essa organela essencial durante a replicação assexuada tem sido uma questão em aberto até o momento. Ancestrais do apicoplasto, como cloroplasto e bactérias, realizam sua distribuição com o auxílio de proteínas da família Min que ainda não foram identificadas em *Plasmodium spp.* Através de intensa pesquisa BLAST, um possível ortólogo de um membro da família Min, MinD, foi identificado em *P. falciparum*. O ortólogo exibe os domínios característicos da função de ATPase descrita para MinD e prevê-se que seja direcionada ao apicoplasto do parasita. A análise da proteína recombinante demonstrou sua capacidade de se ligar ao substrato ATP e polimerizar após adição do substrato. Este efeito é dependente e aprimorado pela adição de metais divalentes. Estudos de localização em *P. falciparum* demonstraram o direcionamento para o apicoplasto. Além disso, a superexpressão do ortólogo nas linhagens de parasitas transgênicos causou um efeito inibitório na proliferação do parasita. Com a ajuda de uma linhagem de referência para visualização do apicoplasto, técnicas de visualização e análise do apicoplasto por imagem de fluorescência de células vivas durante o estágio eritrocítico foram estabelecidas e verificadas, possibilitando a análise da morfologia do apicoplasto sob influência do possível ortólogo de MinD.

Palavres-chave: *Plasmodium falciparum*, apicoplasto, imagens de células vivas, alvo terapêutico.