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Summary

Title of Thesis: Functional and structural characterization of the unique bifunctional enzyme complex involved in regulation of polyamine metabolism in *Plasmodium falciparum*
Student: Lyn-Marie Birkholtz
Supervisor: Prof AI Louw
Co-supervisor: Prof RD Walter
Department: Biochemistry
Degree: *Philosophiae Doctor*

Malaria remains one of the most serious tropical infectious diseases affecting mankind. The prevention of the disease is hampered by the increasing resistance of the parasite to existing chemotherapies. The need for novel therapeutic targets and drugs is therefore of the utmost importance and detailed knowledge of the biochemistry of the parasite is imperative. This study was directed at the biochemical characterisation of the polyamine metabolic pathway of *P. falciparum* in order to elucidate differences between the parasite and its human host that can be exploited in the design of novel antimalarials. The thesis focussed on the two rate-limiting enzymes in polyamine biosynthesis, S-adenosylmethionine decarboxylase (AdoMetDC) and ornithine decarboxylase (ODC), which occur as a unique bifunctional complex in *P. falciparum*.

The genomic structure of the bifunctional gene indicated a single, monocistronic transcript with large untranslated regions that were predicted to be involved in unique translational regulatory mechanisms. This gives rise to a bifunctional protein containing both decarboxylase activities on a single polypeptide forming a heterotetrameric complex. Activity of the decarboxylases decreases dramatically if these proteins are expressed in their monofunctional forms as homodimeric ODC and heterotetrameric AdoMetDC. The deduced amino acid sequence indicated that all the essential residues for catalysis are conserved and highlighted the presence of three parasite-specific insertions.

The parasite-specific inserts were shown to be essential for the catalytic activity of the respective domains and also to influence the activity of the neighbouring domain, indicating that intramolecular communication exists in the heterotetrameric complex. The most structured and smallest insert was also shown to mediate protein-protein interactions between the two domains and to stabilise the complex. Further structure-

functional characterisations of specifically the ODC domain were deduced from a comparative homology model. The model predicted an overall structure corresponding to those of other homologous proteins. The validity of the model is supported by mutagenesis results. However, certain parasite-specific properties were identified in the active site pocket and dimerisation interface. The former was exploited in the rational design of novel putative ODC inhibitors directed only against the *P. falciparum* protein by *in silico* screening of chemical structure libraries.

This study therefore describes the identification of certain parasite-specific properties in a unique bifunctional protein involved in regulation of polyamine metabolism of *P. falciparum*. Such discoveries are invaluable in strategies aimed at elucidating biochemical and metabolic differences between the parasite and its human host that could be exploited in the design of alternative, parasite-specific chemotherapies. Moreover, the thesis also contributed new knowledge on certain less well-understood biological phenomena characteristic of *P. falciparum*, the nature and origin of bifunctional proteins and the functional properties of parasite-specific inserts found in some proteins of the parasite.

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Opsomming

Titel van Tesis: Functional and structural characterization of the unique bifunctional enzyme complex involved in regulation of polyamine metabolism in *Plasmodium falciparum*
Student: Lyn-Marie Birkholtz
Promotor: Prof AI Louw
Medepromotor: Prof RD Walter
Departement: Biochemie
Graad: *Philosophiae Doctor*

Malaria is steeds die mees kommerwekkendste tropiese infeksie wat die mensdom teister. Voorkoming van die siekte word belemmer as gevolg van die parasiete wat weerstandig raak teen die bestaande voorkomende en terapeutiese middels. Dit is dus uiters noodsaaklik om nuwe terapeutiese teikens te identifiseer asook om die biochemiese eienskappe van die parasiet beter te verstaan om sodoende nuwe medisynes te kan ontwerp. Hierdie studie beskryf die biochemiese karakterisering van die poli-amien metaboliese baan van die menslike malaria parasiet, *P. falciparum*, om sodoende verskille tussen die parasiet en sy menslike gasheer te identifiseer wat gebruik kan word in the ontwikkeling van nuwe anti-malaria middels. Twee tempo-beherende ensieme in die baan, S-adenosielmetionien dekarbosilase (AdoMetDC) en ornitien dekarbosilase (ODC), wat voorkom as in 'n unieke, bifunksionele kompleks in *P. falciparum*, was bestudeer.

Die genomiese struktuur van die geen vir die bifunksionele proteïen het aangedui dat `n enkele, monosistroniese transkrip kodeer vir die proteïen. Die groot, ongetransleerde gedeeltes van die transkrip word voorspel om betrokke te wees in alternatiewe regulatoriese meganismes tydens translasie van die proteïen. Die bifunksionele proteïen het dus beide die dekarbosilase aktiwiteite op 'n enkele polipeptied en bestaan as 'n heterotetrameriese kompleks. Die dekarboksilase aktiwiteite verlaag dramaties indien die proteïene uitgedruk word in hul monofunksionele vorme as heterotetrameriese AdoMetDC en homodimeriese ODC. Analises van die afgeleide aminosuurvolgorde van die bifunksionele protein het verskeie gekonserveerde residue aangedui wat essensieel is vir katalitiese aktiwiteit, asook drie parasiet-spesifieke invoegsels.

Die parasiet-spesifieke invoegsels is bewys om essensieël te wees vir die aktiwiteit van die domein waarin dit voorkom maar beïnvloed ook die aktiwiteit van die aangrensende

domein wat die bestaan van intramolekulêre kommunikasie tussen die twee domeine aandui. Die kortste en mees gestruktureerde invoegsel is ook gewys om proteïen-proteïen interaksies te bemiddel om sodoende die bifunksionele kompleks te stabiliseer. Verdere struktuur-funksie karakteriserings van spesifiek die ODC domein is verkry deur `n vergelykende homologie model. Die model voorspel `n algemene struktuur van die proteïen wat gunstig vergelyk met ander homoloë proteïene. Die akkuraatheid van die model is gestaaf deur mutagenese resultate. Verskeie parasiet-spesifieke eienskappe is egter geïdentifiseer beide in die aktiewe setel sowel as in die dimerisasie intervlak van die proteïen. Die voorspelde struktuur van die aktiewe setel is verder gebruik in die identifikasie van nuwe, vermoedelike spesifieke inhibitore van *P. falciparum* ODC deur *in silico* sifting van chemiese struktuur biblioteke.

Hierdie studie beskryf dus verskeie parasiet-spesifieke eienskappe van die unieke bifunksionele proteïen wat poli-amien metabolisme van *P. falciparum* reguleer. Sulke ontdekkings is waardevol in strategieë wat fokus op biochemiese en metaboliese verskille tussen die parasiet en sy menslike gasheer om sodoende alternatiewe, parasiet-spesifieke chemoterapeutiese middels te ontwikkel. Verder dra hierdie studie ook nuwe kennis by tot ander biologiese aspekte van *P. falciparum* insluitend die oorsprong en karakter van bifunksionele proteïene asook die funksionele bydrae van die parasiet-spesifieke invoegsels in sommige proteïene van die parasiet.

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Appendix I: Multiple alignment of the genomic DNA and cDNA sequences of *PfAdometdc/Odc*.

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PfA/O cDNA : ----------AAAAAAAAAAAAAAAAAATATAGATCCATATATCGAAATTATCCCTATATCTTAACATCTCTAATAATG :   69
cDNA       : ----------------------------------------------------------------------------ATG :    3
PfA/O gDNA : CAATCTTTACCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAATATAGATCCATATATCGAAATTATCCCTATATCTTAACATCTCTAATAATG : 2184
gDNA       : ----------------------------------------------------------------------------ATG :    3

PfA/O cDNA : AACGGAATTTTTGAAGGAATTGAAAAAAGGGTTGTGATCAAATTAAAGGAGAGTTTTTTCAAAGGAAATAGAAATGTGAACTCCTTTTTAG :  160
cDNA       : AACGGAATTTTTGAAGGAATTGAAAAAAGGGTTGTGATCAAATTAAAGGAGAGTTTTTTCAAAGGAAATAGAAATGTGAACTCCTTTTTAG :   94
PfA/O gDNA : AACGGAATTTTTGAAGGAATTGAAAAAAGGGTTGTGATCAAATTAAAGGAGAGTTTTTTCAAAGGAAATAGAAATGTGAACTCCTTTTTAG : 2275
gDNA       : AACGGAATTTTTGAAGGAATTGAAAAAAGGGTTGTGATCAAATTAAAGGAGAGTTTTTTCAAAGGAAATAGAAATGTGAACTCCTTTTTAG :   94
                 Sampetf1

PfA/O cDNA : ATATACCTAAAGAATTATGGGAAGAAAAATTAAAATACATTGGTTGTAGTATTGTATCGGAAATAAGTGAGGACAAGAACGAGAGAAGAGG :  251
cDNA       : ATATACCTAAAGAATTATGGGAAGAAAAATTAAAATACATTGGTTGTAGTATTGTATCGGAAATAAGTGAGGACAAGAACGAGAGAAGAGG :  185
PfA/O gDNA : ATATACCTAAAGAATTATGGGAAGAAAAATTAAAATACATTGGTTGTAGTATTGTATCGGAAATAAGTGAGGACAAGAACGAGAGAAGAGG : 2366
gDNA       : ATATACCTAAAGAATTATGGGAAGAAAAATTAAAATACATTGGTTGTAGTATTGTATCGGAAATAAGTGAGGACAAGAACGAGAGAAGAGG :  185

PfA/O cDNA : TGAACGATGTCGTGTGTATTTATTGTCAGAGAGTTCTTTATACATTTTTGATGATTCTTTATTTATTAAGACATGTGGCAAAACAAGAGTT :  342
cDNA       : TGAACGATGTCGTGTGTATTTATTGTCAGAGAGTTCTTTATACATTTTTGATGATTCTTTATTTATTAAGACATGTGGCAAAACAAGAGTT :  276
PfA/O gDNA : TGAACGATGTCGTGTGTATTTATTGTCAGAGAGTTCTTTATACATTTTTGATGATTCTTTATTTATTAAGACATGTGGCAAAACAAGAGTT : 2457
gDNA       : TGAACGATGTCGTGTGTATTTATTGTCAGAGAGTTCTTTATACATTTTTGATGATTCTTTATTTATTAAGACATGTGGCAAAACAAGAGTT :  276

PfA/O cDNA : TTATTTTTCATACCGTTTGTGGTTGATTTATTAATATATCATATGGATAATGTAGGTATAATAGAAAAGAATTGTGTATATGATGAGACGT :  433
cDNA       : TTATTTTTCATACCGTTTGTGGTTGATTTATTAATATATCATATGGATAATGTAGGTATAATAGAAAAGAATTGTGTATATGATGAGACGT :  367
PfA/O gDNA : TTATTTTTCATACCGTTTGTGGTTGATTTATTAATATATCATATGGATAATGTAGGTATAATAGAAAAGAATTGTGTATATGATGAGACGT : 2548
gDNA       : TTATTTTTCATACCGTTTGTGGTTGATTTATTAATATATCATATGGATAATGTAGGTATAATAGAAAAGAATTGTGTATATGATGAGACGT :  367

PfA/O cDNA : TTATTGAAAACGAGAAATTCCATAATATAGCTGAATTCATAAAAGAACATTTCCTTTATTGTTTTTTTACACATATGAATTACCGAAATAA :  524
cDNA       : TTATTGAAAACGAGAAATTCCATAATATAGCTGAATTCATAAAAGAACATTTCCTTTATTGTTTTTTTACACATATGAATTACCGAAATAA :  458
PfA/O gDNA : TTATTGAAAACGAGAAATTCCATAATATAGCTGAATTCATAAAAGAACATTTCCTTTATTGTTTTTTTACACATATGAATTACCGAAATAA : 2639
gDNA       : TTATTGAAAACGAGAAATTCCATAATATAGCTGAATTCATAAAAGAACATTTCCTTTATTGTTTTTTTACACATATGAATTACCGAAATAA :  458

PfA/O cDNA : AACAAAGGATGGTTATTTTGAACAGGAATATCCACACAAATCTCTTGAAGATGAAAAGAAATTTTTTGAGTTTTTTTTTTAAGAACGTACAA :  615
cDNA       : AACAAAGGATGGTTATTTTGAACAGGAATATCCACACAAATCTCTTGAAGATGAAAAGAAATTTTTTGAGTTTTTTTTTTAAGAACGTACAA :  549
PfA/O gDNA : AACAAAGGATGGTTATTTTGAACAGGAATATCCACACAAATCTCTTGAAGATGAAAAGAAATTTTTTGAGTTTTTTTTTTAAGAACGTACAA : 2730
gDNA       : AACAAAGGATGGTTATTTTGAACAGGAATATCCACACAAATCTCTTGAAGATGAAAAGAAATTTTTTGAGTTTTTTTTTTAAGAACGTACAA :  549

PfA/O cDNA : ATGTATAATACACATTTACCTATGGAGAAAATGCATTATATATTCTTCTACTCTTCTGATGATGTACATATGACGGATATAGCTTCTACGT :  706
cDNA       : ATGTATAATACACATTTACCTATGGAGAAAATGCATTATATATTCTTCTACTCTTCTGATGATGTACATATGACGGATATAGCTTCTACGT :  640
PfA/O gDNA : ATGTATAATACACATTTACCTATGGAGAAAATGCATTATATATTCTTCTACTCTTCTGATGATGTACATATGACGGATATAGCTTCTACGT : 2821
gDNA       : ATGTATAATACACATTTACCTATGGAGAAAATGCATTATATATTCTTCTACTCTTCTGATGATGTACATATGACGGATATAGCTTCTACGT :  640

PfA/O cDNA : TTAAATTCTGTTCGGAAATACATTTGTTTGGAATTAACAAATATAATGAAAAGAATCAATTCCATGACGCTTATCTGAATAACAAGTCGTT :  797
cDNA       : TTAAATTCTGTTCGGAAATACATTTGTTTGGAATTAACAAATATAATGAAAAGAATCAATTCCATGACGCTTATCTGAATAACAAGTCGTT :  731
PfA/O gDNA : TTAAATTCTGTTCGGAAATACATTTGTTTGGAATTAACAAATATAATGAAAAGAATCAATTCCATGACGCTTATCTGAATAACAAGTCGTT : 2912
gDNA       : TTAAATTCTGTTCGGAAATACATTTGTTTGGAATTAACAAATATAATGAAAAGAATCAATTCCATGACGCTTATCTGAATAACAAGTCGTT :  731

PfA/O cDNA : GAATCTATTTACAAGAGTACATGAGGATAATTTAAAGCTTTATGATAGTAGTGATGCTGATAAGGAAGTAACCACCCACATCTATAGTACC :  888
cDNA       : GAATCTATTTACAAGAGTACATGAGGATAATTTAAAGCTTTATGATAGTAGTGATGCTGATAAGGAAGTAACCACCCACATCTATAGTACC :  822
PfA/O gDNA : GAATCTATTTACAAGAGTACATGAGGATAATTTAAAGCTTTATGATAGTAGTGATGCTGATAAGGAAGTAACCACCCACATCTATAGTACC : 3003
gDNA       : GAATCTATTTACAAGAGTACATGAGGATAATTTAAAGCTTTATGATAGTAGTGATGCTGATAAGGAAGTAACCACCCACATCTATAGTACC :  822

PfA/O cDNA : AGAGGGACATATGAAGATACAGGAATGGTGAATTGTGTGGATGTGATTTATAAGAATGAAAGTACATTGTTAAATAGGAATAATATAGAAA :  979
cDNA       : AGAGGGACATATGAAGATACAGGAATGGTGAATTGTGTGGATGTGATTTATAAGAATGAAAGTACATTGTTAAATAGGAATAATATAGAAA :  913
PfA/O gDNA : AGAGGGACATATGAAGATACAGGAATGGTGAATTGTGTGGATGTGATTTATAAGAATGAAAGTACATTGTTAAATAGGAATAATATAGAAA : 3094
gDNA       : AGAGGGACATATGAAGATACAGGAATGGTGAATTGTGTGGATGTGATTTATAAGAATGAAAGTACATTGTTAAATAGGAATAATATAGAAA :  913

PfA/O cDNA : ATATTCCATCTATTGAAAATAAAGAAAGTAATAATAATAGTAGATGTTGTCATAATAATAATTATAGTGGAAGTTGTCATAATATTGTGAG : 1070
cDNA       : ATATTCCATCTATTGAAAATAAAGAAAGTAATAATAATAGTAGATGTTGTCATAATAATAATTATAGTGGAAGTTGTCATAATATTGTGAG : 1004
PfA/O gDNA : ATATTCCATCTATTGAAAATAAAGAAAGTAATAATAATAGTAGATGTTGTCATAATAATAATTATAGTGGAAGTTGTCATAATATTGTGAG : 3185
gDNA       : ATATTCCATCTATTGAAAATAAAGAAAGTAATAATAATAGTAGATGTTGTCATAATAATAATTATAGTGGAAGTTGTCATAATATTGTGAG : 1004

PfA/O cDNA : TGTTGTTCCTTCCGAAAGAAATAATGATCATGTACATCACAGACATTATGAAGATACCTTAAATCGTTCTAATATTTCTGCTGAAGATAAC : 1161
cDNA       : TGTTGTTCCTTCCGAAAGAAATAATGATCATGTACATCACAGACATTATGAAGATACCTTAAATCGTTCTAATATTTCTGCTGAAGATAAC : 1095
PfA/O gDNA : TGTTGTTCCTTCCGAAAGAAATAATGATCATGTACATCACAGACATTATGAAGATACCTTAAATCGTTCTAATATTTCTGCTGAAGATAAC : 3276
gDNA       : TGTTGTTCCTTCCGAAAGAAATAATGATCATGTACATCACAGACATTATGAAGATACCTTAAATCGTTCTAATATTTCTGCTGAAGATAAC : 1095

PfA/O cDNA : AATAGAAATGCACAACCAGAAAAAGAAAAGGACGAAGATGTAAGAAGAGATGATGAAGAAAATAAAGTTCTAATAAAAATGATAGATACGA : 1252
cDNA       : AATAGAAATGCACAACCAGAAAAAGAAAAGGACGAAGATGTAAGAAGAGATGATGAAGAAAATAAAGTTCTAATAAAAATGATAGATACGA : 1186
PfA/O gDNA : AATAGAAATGCACAACCAGAAAAAGAAAAGGACGAAGATGTAAGAAGAGATGATGAAGAAAATAAAGTTCTAATAAAAATGATAGATACGA : 3367
gDNA       : AATAGAAATGCACAACCAGAAAAAGAAAAGGACGAAGATGTAAGAAGAGATGATGAAGAAAATAAAGTTCTAATAAAAATGATAGATACGA : 1186

PfA/O cDNA : ATTTATACGAATGTATAAATTATAATAAGGAAAGCTTTTTATATAATGAATTTTATTTTACACCTTGTGGTTATTCTTGTAATGTTTCTGA : 1343
cDNA       : ATTTATACGAATGTATAAATTATAATAAGGAAAGCTTTTTATATAATGAATTTTATTTTACACCTTGTGGTTATTCTTGTAATGTTTCTGA : 1277
PfA/O gDNA : ATTTATACGAATGTATAAATTATAATAAGGAAAGCTTTTTATATAATGAATTTTATTTTACACCTTGTGGTTATTCTTGTAATGTTTCTGA : 3458
gDNA       : ATTTATACGAATGTATAAATTATAATAAGGAAAGCTTTTTATATAATGAATTTTATTTTACACCTTGTGGTTATTCTTGTAATGTTTCTGA : 1277

PfA/O cDNA : AAAAAATAATTATTTTTGTGTACATTATTCACCAGAAGATTCTGTATCGTACGTTTCTGTTGAGGTATCTTCAAATTTGTCGTGTGATCGA : 1434
cDNA       : AAAAAATAATTATTTTTGTGTACATTATTCACCAGAAGATTCTGTATCGTACGTTTCTGTTGAGGTATCTTCAAATTTGTCGTGTGATCGA : 1368
PfA/O gDNA : AAAAAATAATTATTTTTGTGTACATTATTCACCAGAAGATTCTGTATCGTACGTTTCTGTTGAGGTATCTTCAAATTTGTCGTGTGATCGA : 3549
gDNA       : AAAAAATAATTATTTTTGTGTACATTATTCACCAGAAGATTCTGTATCGTACGTTTCTGTTGAGGTATCTTCAAATTTGTCGTGTGATCGA : 1368
                                                  Samdcd1

PfA/O cDNA : TTTTTAGACTTTATTCACAAGCAGTTGAATTTTTATAATGGAAAGTATATGTTCATGATAAATTATGTATTCTGTGAGGAGAGTAACAACA : 1525
cDNA       : TTTTTAGACTTTATTCACAAGCAGTTGAATTTTTATAATGGAAAGTATATGTTCATGATAAATTATGTATTCTGTGAGGAGAGTAACAACA : 1459
PfA/O gDNA : TTTTTAGACTTTATTCACAAGCAGTTGAATTTTTATAATGGAAAGTATATGTTCATGATAAATTATGTATTCTGTGAGGAGAGTAACAACA : 3640
gDNA       : TTTTTAGACTTTATTCACAAGCAGTTGAATTTTTATAATGGAAAGTATATGTTCATGATAAATTATGTATTCTGTGAGGAGAGTAACAACA : 1459
                                          ODCf1                ODCexpf1

PfA/O cDNA : TGTCTAAAATGGTACCTGATGATGATAATAATAATTATAGTAGTGGTAAAAGTTGCGTTTATTATCAAGATTTAAATAAGAAAGAAAAAGA : 1616
cDNA       : TGTCTAAAATGGTACCTGATGATGATAATAATAATTATAGTAGTGGTAAAAGTTGCGTTTATTATCAAGATTTAAATAAGAAAGAAAAAGA : 1550
PfA/O gDNA : TGTCTAAAATGGTACCTGATGATGATAATAATAATTATAGTAGTGGTAAAAGTTGCGTTTATTATCAAGATTTAAATAAGAAAGAAAAAGA : 3731
gDNA       : TGTCTAAAATGGTACCTGATGATGATAATAATAATTATAGTAGTGGTAAAAGTTGCGTTTATTATCAAGATTTAAATAAGAAAGAAAAAGA : 1550
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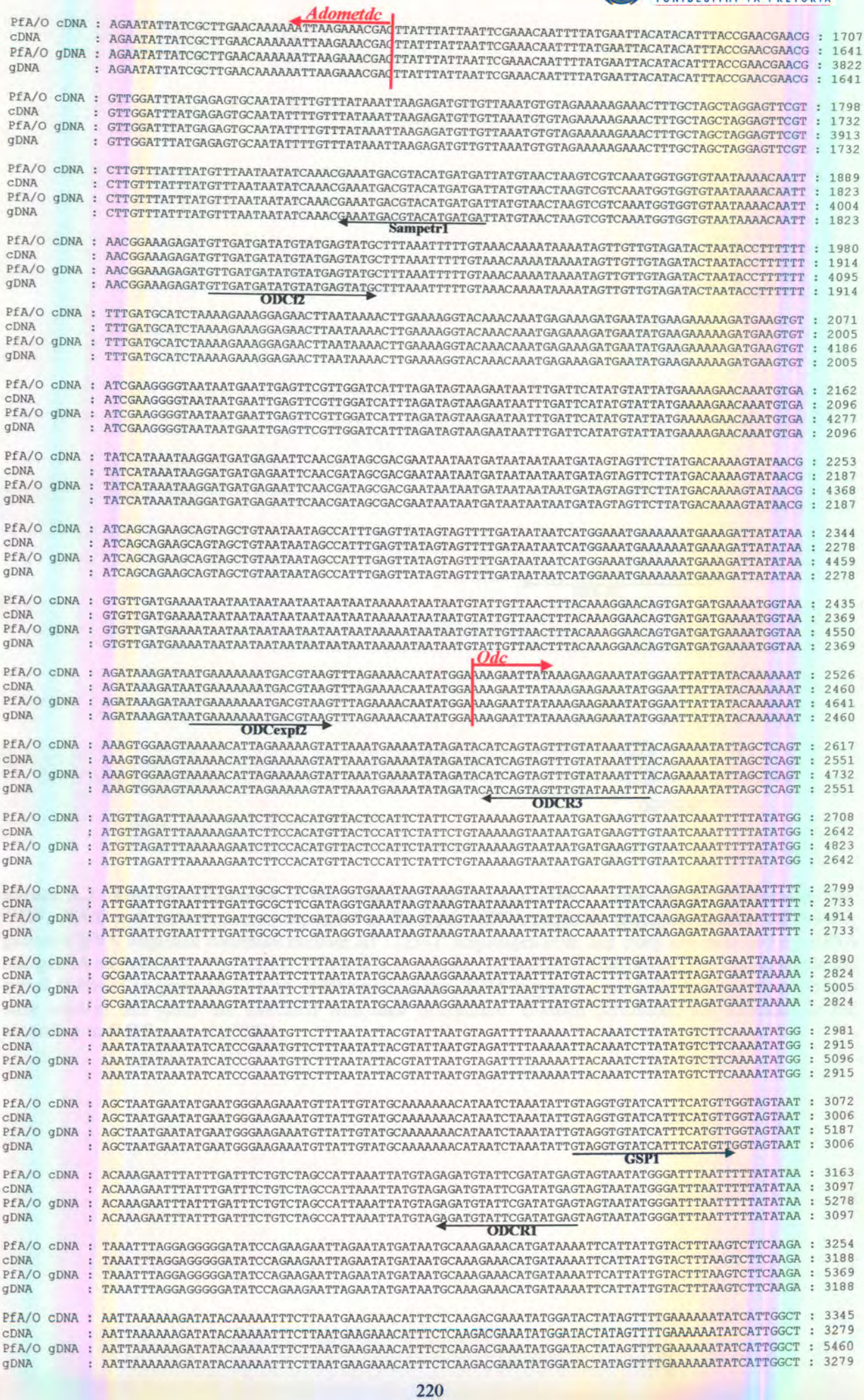
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Sampetr1
ODCt2
Odc
ODCexpt2
ODCR3
GSPT
ODCR1

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cDNA       : ATTAATATGTCAATCGATCATTATTTTAGTCATATGAAAGATAATCTAAGAGTTATTTGTGAACCTGGTAGATATATGGTCGCTGCTTCGT : 3370
PfA/O gDNA : ATTAATATGTCAATCGATCATTATTTTAGTCATATGAAAGATAATCTAAGAGTTATTTGTGAACCTGGTAGTAATATGGTCGCTGCTTCGT : 5551
gDNA       : ATTAATATGTCAATCGATCATTATTTTAGTCATATGAAAGATAATCTAAGAGTTATTTGTGAACCTGGTAGTAATATGGTCGCTGCTTCGT : 3370

PfA/O cDNA : CAACATTAGCTGTTAAAATTATAGGAAAGAGACGTCCAACTTTTCAGGGCATTATGTTAAAAGAATTAAAAGACCATTACGATCCTTTAAA : 3527
cDNA       : CAACATTAGCTGTTAAAATTATAGGAAAGAGACGTCCAACTTTTCAGGGCATTATGTTAAAAGAATTAAAAGACCATTACGATCCTTTAAA : 3461
PfA/O gDNA : CAACATTAGCTGTTAAAATTATAGGAAAGAGACGTCCAACTTTTCAGGGCATTATGTTAAAAGATTTAAAAGACCATTACGATCCTTTAAA : 5642
gDNA       : CAACATTAGCTGTTAAAATTATAGGAAAGAGACGTCCAACTTTTCAGGGCATTATGTTAAAAGATTTAAAAGACCATTACGATCCTTTAAA : 3461

PfA/O cDNA : TTTTGCTCAACAAGAAAATAAGAAACAAGACGAAACAAAAATAAACCACAATAATGATAATAATGATAATAATGATAATAATGATAATAAT : 3618
cDNA       : TTTTGCTCAACAAGAAAATAAGAAACAAGACGAAACAAAAATAAACCACAATAATGATAATAAaGtaatgaaaTaATAATAATGATAATAAT : 3552
PfA/O gDNA : TTTTGCTCCACAAGAAAATAAGAAACAAGACGAAACAAAAATAAACCACAATAATGATAATAATGATAATAATGATAATAATGATAATAAT : 5733
gDNA       : TTTTGCTCCACAAGAAAATAAGAAACAAGACGAAACAAAAATAAACCACAATAATGATAATAATGATAATAATGATAATAATGATAATAAT : 3552

PfA/O cDNA : ATTAATAATAATAATAATAATCAAAAAGGGGGCCAAGGAAATATTATGAATGATCTAATAATAACTAGCACAAATGATTCTACTAGTAAAA : 3709
cDNA       : ATTAATAATAATAATAATAATCAAAAAGGGGGCCAAGGAAATATTATGAATGATCTAATAATAACTAGCACAAATGATTCTACTAGTAAAA : 3643
PfA/O gDNA : ATTAATAATAATAATAATAATCAAAAAGGGGGCCAAGGAAATATTATGAATGATCTAATAATAACTAGCACAAATGATTCTACTAGTAAAA : 5824
gDNA       : ATTAATAATAATAATAATAATCAAAAAGGGGGCCAAGGAAATATTATGAATGATCTAATAATAACTAGCACAAATGATTCTACTAGTAAAA : 3643

PfA/O cDNA : AGAATGATCATTCTTCTAGTCAAGTTATTCAAAATGTATCGTGCACAATACGTGATAAAGAAGGAGATAATATTAAAATAAATACACATAC : 3800
cDNA       : AGAATGATCATTCTTCTAGTCAAGTTATTCAAAATGTATCGTGCACAATACGTGATAAAGAAGGAGATAATATTAAAATAAATACACATAC : 3734
PfA/O gDNA : AGAATGATCATTCTTCTAGTCAAGTTATTCAAAATGTATCGTGCACAATACGTGATAAAGAAGGAGATAATATTAAAATAAATACACATAC : 5915
gDNA       : AGAATGATCATTCTTCTAGTCAAGTTATTCAAAATGTATCGTGCACAATACGTGATAAAGAAGGAGATAATATTAAAATAAATACACATAC : 3734

PfA/O cDNA : CATAAATAATCCTAATATAAATGGAAAAGAAAATACCGTGGATGGTGATAATATTAATATTGCTCATAAAAATATTGGTAATAACTTTAGT : 3891
cDNA       : CATAAATAATCCTAATATAAATGGAAAAGAAAATACCGTGGATGGTGATAATATTAATATTGCTCATAAAAATATTGGTAATAACTTTAGT : 3825
PfA/O gDNA : CATAAATAATCCTAATATAAATGGAAAAGAAAATACCGTGGATGGTGATAATATTAATATTGCTCATAAAAATATTGGTAATAACTTTAGT : 6006
gDNA       : CATAAATAATCCTAATATAAATGGAAAAGAAAATACCGTGGATGGTGATAATATTAATATTGCTCATAAAAATATTGGTAATAACTTTAGT : 3825

PfA/O cDNA : AGTAGTAATTCAAAATTAGGCAACATAACAAATATTAAGAAAAAAGTTGTTAATATTAATGACAATAGATATAATTATTTCTCATATTATG : 3982
cDNA       : AGTAGTAATTCAAAATTAGGCAACATAACAAATATTAAGAAAAAAGTTGTTAATATTAATGACAATAGATATAATTATTTCTCATATTATG : 3916
PfA/O gDNA : AGTAGTAATTCAAAATTAGGCAACATAACAAATATTAAGAAAAAAGTTGTTAATATTAATGACAATAGATATAATTATTTCTCATATTATG : 6097
gDNA       : AGTAGTAATTCAAAATTAGGCAACATAACAAATATTAAGAAAAAAGTTGTTAATATTAATGACAATAGATATAATTATTTCTCATATTATG : 3916

PfA/O cDNA : TAAGCGATAGTATATATGGTTGTTTTAGTGGTATAATTTTTGATGAATACAATAGATGTCCTATTTATGTTATTAAAAACAAAAATAACCC : 4073
cDNA       : TAAGCGATAGTATATATGGTTGTTTTAGTGGTATAATTTTTGATGAATACAATAGATGTCCTATTTATGTTATTAAAAACAAAAATAACCC : 4007
PfA/O gDNA : TAAGCGATAGTATATATGGTTGTTTTAGTGGTATAATTTTTGATGAATACAATAGATGTCCTATTTATGTTATTAAAAACAAAAATAACCC : 6188
gDNA       : TAAGCGATAGTATATATGGTTGTTTTAGTGGTATAATTTTTGATGAATACAATAGATGTCCTATTTATGTTATTAAAAACAAAAATAACCC : 4007
                                                                                  ODCR2
PfA/O cDNA : TAATCAAAATTTTATGAATTTTAATTTGTATTTAGCTAATGTATTTGGACAATCATGTGATGGCTTGGATATGATCAATTCTATTACGTAC : 4164
cDNA       : TAATCAAAATTTTATGAATTTTAATTTGTATTTAGCTAATGTATTTGGACAATCATGTGATGGCTTGGATATGATCAATTCTATTACGTAC : 4098
PfA/O gDNA : TAATCAAAATTTTATGAATTTTAATTTGTATTTAGCTAATGTATTTGGACAATCATGTGATGGCTTGGATATGATCAATTCTATTACGTAC : 6279
gDNA       : TAATCAAAATTTTATGAATTTTAATTTGTATTTAGCTAATGTATTTGGACAATCATGTGATGGCTTGGATATGATCAATTCTATTACGTAC : 4098

PfA/O cDNA : TTACCTGAGTGTTATATTAATGATTGGCTTCTCTATGAATATGCTGGGGCATACACTTTTGTCAGCTCATCAAACTTTAATGGATTTAAGA : 4255
cDNA       : TTACCTGAGTGTTATATTAATGATTGGCTTCTCTATGAATATGCTGGGGCATACACTTTTGTCAGCTCATCAAACTTTAATGGATTTAAGA : 4189
PfA/O gDNA : TTACCTGAGTGTTATATTAATGATTGGCTTCTCTATGAATATGCTGGGGCATACACTTTTGTCAGCTCATCAAACTTTAATGGATTTAAGA : 6370
gDNA       : TTACCTGAGTGTTATATTAATGATTGGCTTCTCTATGAATATGCTGGGGCATACACTTTTGTCAGCTCATCAAACTTTAATGGATTTAAGA : 4189

PfA/O cDNA : AATGCAAGAAGGTGTATATATTCCCTGAATCGAAACCTTCCCTTAAGGGGCAACCAAACAAACATTGGTAAATAACAAAATCGAAGAAAAA : 4346
cDNA       : AATGCAAGAAGGTGTATATATTCCCTGAATCGAAACCTTCCCTTAAGGGGCAACCAAACAAACATTGGTAAATAACAAAATCGAAGAAAAA : 4280
PfA/O gDNA : AATGCAAGAAGGTGTATATATTCCCTGAATCGAAACCTTCCCTTAAGGGGCAACCAAACAAACATTGGTAAATAACAAAATCGAAGAAAAA : 6461
gDNA       : AATGCAAGAAGGTGTATATATTCCCTGAATCGAAACCTTCCCTTAAGGGGCAACCAAACAAACATTGG---------------------- : 4257
                                                                                  ODCexpr
PfA/O cDNA : GGAAATAAATAGGGAAAAAAAAAAAAAAAAAAAAAAA---------------------------------------------------- : 4383
cDNA       : GGAAATAAATAGGGAAAAAAAAAAAAAAAAAAA---------------------------------------------------------- :4312
PfA/O gDNA : GGAAATAAATAGGGAAAAAAAAAAAAAAAAAAAGAAAAAAAAAAAAAAAGAAAAAAAAAAAAAAAGAAAAAGAAAAAAAAAAAAAGAAAAAGAAAA : 6552
gDNA       : ----------------------------------------------------------------------------------------- :    -
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Figure A.1: Multiple-alignment of the genomic (gDNA) and cDNA sequences of *PfAdometdc/Odc* ORF. PfA/O indicates sequences deposited in Genbank: cDNA sequence accession number AF094833, genomic DNA sequence accession number AF112367. Sequences from this study are indicated by cDNA and gDNA respectively. The start (ATG) and stop codons (TAA) are in blue boxes. The poly-adenylation signal is boxed in green (AATAA). Differences in the cDNA sequences are indicated in yellow. Primer sites used in this study are indicated with their orientation. Domain definitions are indicated in red according to (Müller, *et al.*, 2000).