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Transcription Profiles of *Eimeria tenella* Apical Membrane Antigen Gene, *Etama*

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Abstract: The invasion of Apicomplaxan parasite *Eimeria tenella* is a multistep process, after attaching the host cell, the tight junction complex is formed by proteins secreted from apical organelles, rhoptry (RONs) and microneme (Apical Membrane Antigen, AMA) between the parasite and host cell plasma membranes which is named as Moving Juction (MJ) and through which the parasite pulls itself into the host cell. In this study, researchers have used real-time quantitative RT-PCR to study the transcription profiles of *Etama* gene for the 1st time on eimeria parasites. The results showed that the transcription of *Etama* gene reached a peak during the sporozoites stage but it was very low during all other stages which is different from other Apicomplex parasites *Etama1* gene. So maybe there are other AMA family proteins in the *Eimeria tenella* which play a key role in the different stages, respectively.

Key words: *Eimeria tenella*, apical membrane antigen, real-time quantitative RT-PCR, transcription, stages

INTRODUCTION

Apicomplaxan parasite *Eimeria tenella* is an obligate intracellular parasite which causes avian coccidiosis and shares largely conserved invasion mechanism with other Apicomplaxan parasites including *Toxoplasma gondii* and the malaria parasite *Plasmodium falciparum*, etc. (Levine, 1988). It causes 30-100% morbidity and 80% mortality in the case of no any measures on the prevention (Dalloul and Lillehoj, 2006). Recent date indicate that about £1.5 billion global costs annually (Shirley *et al*., 2005).

The apicomplxan parasites have a highly conserved invasion mechanism which is a multistep process (Dubremetz *et al*., 1998). After attaching the host cell, some invasion-associated proteins are secreted from apical secretory organelles including MICs, AMA1, ROPs and RONs, etc. (Cerede *et al*., 2005; Igonet *et al*., 2006; Nichols *et al*., 1983; El-Hajj *et al*., 2007). After being mediated by these proteins, the parasites penetrate into host cell and establish the parasitic life in a membrane-bound structure called the Parasitophorous Vacuole (PV) (Mercier *et al*., 2005).

Apical Membrane Antigen (AMA) is a kind of transmembrane protein which is identified as a conserved antigenic protein in the aipcomplexan parasites (Beghetto *et al*., 2005). Recently, lots of studies have being reported about the function of AMA and they found that AMA was localized to the entire surfaces of extracellular parasites and to the surface of host cell just prior to invasion (Richard *et al*., 2010).

It can form a ring structure with rhoptry neck proteins during invasion which is termed Moving Juction (MJ), a tight connection between parasite and the membranes of host cell and the parasite penetrate into host cell through this ring (Straub *et al*., 2009). So, the AMA plays a very important role during the parasite invasion but there are limited reports about AMA in *Eimeria tellena*.

In this study, to clone *Eimeria tenella* apical membrane antigen *Etama* gene and study the transcription profiles of *Etama* gene during the different development stages of *Eimeria tenella*. The sequence encoding Etama protein were amplified by RT-PCR from *Eimeria tenella* (Guangdong strain) total RNA template. The parasites at different developmental stages were harvested and their total RNA were substracted by real-time PCR using *Etactin* gene as a reference for establishing standard curves to evaluate the copy number of *Etama* genes.

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MATERIALS AND METHODS

Parasites and preparetion of template cDNA: The fresh *Eimeria tenella* oocysts (Guangdong strain) were used in all experiments. Oocysts, second generation merozoites and sporozoites were purified as described previously (Hofmann and Raether, 1990; Xie *et al*., 1994). Total RNA was isolated using TRIzol® Reagent (Invitrogen acquires Life Technologies Inc.) RNA isolation protocol and treated with DNase (Promega). The first strand cDNA of samples was synthesized from the samples total RNA in different stages with PrimeScriptTM RT reagent kit Perfect Real-time (TAKaRa).

Real-time quantitative transcript analysis: Transcription of Etama was evaluated in the different stages of *Eimeria tenella* by quantitative real-time Reverse Transcription-PCR (RT-PCR) using SYBR® Premix Ex Taq™ (TaKara) and a LightCycle 1.5 System (Roche Diagnostics) and 1 μL cDNA was used in each 20 μL reaction, three replicate reactions were performed for each sample. As a control, transcription of Etactin was also evaluated. Five sporozoite cDNA samples were prepared by 10 fold Serial Dilution Method which was used to make a standard curve to evaluate the amplification Efficacy (E) of *Etactin* and *Etama* gene. The specific primers were created using the Primer Premier 5.0 for the Etama (product size is 125 bp) and Etactin (product size is 305 bp): F_{actin}: 5'-CACCACCGCCGAGAAAGA-3' R_{actin}: 5'-GAACAACAT TGCCGTAGAGG-3';F_{ama}:5'-CACATTTCCCGCCTCCCT-3'; R_{ama}: 5'-CGCACTTGCTGCCGTCTT-3' and the primer concentration were optimized by spanning the terminal concentration range of 100 nM to 2 μM using conventional PCR. The date analysis was carried out with following equation:

$$Ratio_{(test/refrence)} = E_{refrence}^{\Delta CT}/E_{test}^{\Delta CT}$$

RESULTS AND DISCUSSION

The optimal primer concentration for real time qRT-PCR: The optimal primer concentrations were 500 nM, respectively in which condition, there was only peculiar PCR amplification product was identified by PCR and there was no non-peculiar band and the primer dimmers (Fig. 1).

Generation of standard curve: The standard curves were drawn according to the results of amplification of standard samples (Fig. 2) and the amplification efficiencies for Etactin and Etama of the real-time quantitative PCR Method were 2.03 and 1.93, respectively which showed that the genes were well amplified under this condition.

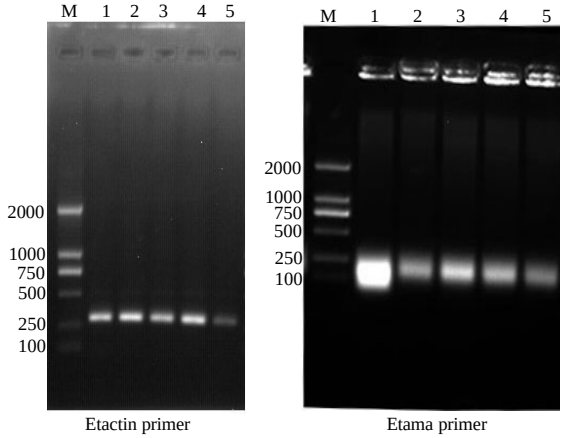

Fig. 1: Optimization of primer concentration for real time qRT-PCR; the primer concentration were optimized by spanning the terminal concentration range of 100 nM to 2 μM using conventional PCR. The optimal primer concentrations were 500 nM, respectively; M: DNA Marker DL 2000; 1:2 and 2:1 μM; 3:500; 4:250 and 5:100 nM

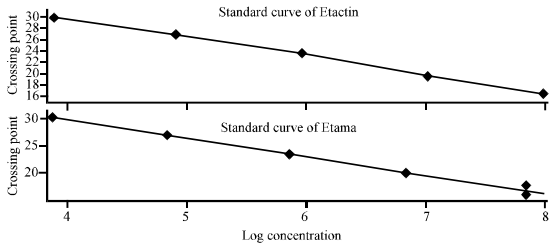

Fig. 2: The sample standard curve in real time q-PCR; five sporozoite cDNA samples were prepared by 10 fold Serial Dilution Method which was used to make a standard curve to evaluate the amplification Efficacy (E) of *Etactin* and *Etama* gene. The amplification efficiencies for Etactin and Etama of the real-time quantitative PCR Method were 2.03 and 1.93, respectively

Analysis of Etama transcription: The Etama transcription peaks at the sporozoite, the Etactin transcription as a control (Fig. 3) and it was very low during all other stages which is different from other apicomplex parasites *Etama1* gene. In this study, we characterized the transcription of Etama in various developmental stages and we found the transcription of *Etama* gene was the highest regulated at the sporozoite stage. It was first time to use real-time quantitative PCR Method for analysis of the *Eimeria tellena* gene transcription in various developmental stages and the method proved to be a highly effective way to study the dynamic changes of interesting genes transcription.

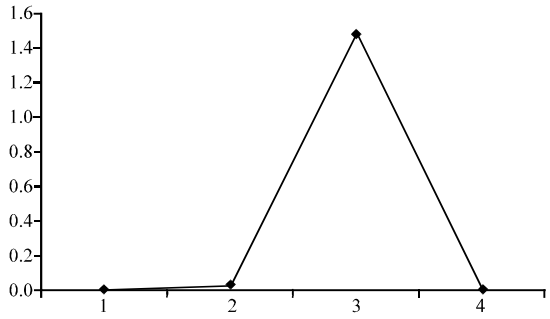

Fig. 3: The transcription of Etama in various developmental stages. The Etama transcription peaks at the sporozoite, the Etactin transcription as a control; 1: unsporulated oocysts; 2: sporulated oocysts; 3: sporozoites; 4: merozoites

In the previous study of other apicomplexan protozoa, Apical Membrane Antigen 1 (AMA1) and Rhoptry Neck proteins (RON2, RON4, RON5 and RON8) were thought to interact with each other as a complex which was named as Moving Juction (MJ) and involved in parasite invasion of host cells (Cao *et al*., 2009; Alexander *et al*., 2006; Proellocks *et al*., 2009; Straub *et al*., 2011). For *Eimeria tenella* parasites, there are two important invasion stages: sporozoite and merozoite stages. But the dates in this study showed that the *Etama* gene transcription peaked only at the sporozoite stage, it was very low in the merozoite stage and *Etron2* gene transcription was similar in these two stages which demonstrated either Etama protein maybe not necessary for parasite invasion of host cells or there were some other proteins played a similar role as Etama in the merozoite stage.

CONCLUSION

In this study combining the results of Proteomic comparison of four *Eimeria tenella* life cycle stages which found there were two Etama proteins, Etama1, Etama2 and Etama1 was found only in merozoites, Etama2 was only found in sporozoites (Lal *et al*., 2009), we can presume that Etama is necessary for *Eimeria tenella* parasites invasion and there are different kinds of Etama proteins play a similar role in different invasion stages.

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REFERENCES

Alexander, D.L., S. Arastu-Kapur, J.F. Dubremetz and J.C. Boothroyd, 2006. *Plasmodium falciparum* AMA1 binds a rhoptry neck protein homologous to TgRON4, a component of the moving junction in *Toxoplasma gondii*. Eukaryot. Cell, 5: 1169-1173.

Beghetto, E., H.V. Nielsen, P. Del Porto, W. Buffolano and S. Guglietta, 2005. A combination of antigenic regions of *Toxoplasma gondii* microneme proteins induces protective immunity against oral infection with parasite cysts. J. Infect. Dis., 191: 637-645.

Cao, J., O. Kaneko, A. Thongkukiatkul, M. Tachibana and H. Otsuki *et al*., 2009. Rhoptry neck protein RON2 forms a complex with microneme protein AMA1 in *Plasmodium falciparum* merozoites. Parasitol. Int., 58: 29-35.

Cerede, O., J.F. Dubremetz, M. Soete, D. Deslee, H. Vial, D. Bout and M. Lebrun, 2005. Synergistic role of micronemal proteins in *Toxoplasma gondii* virulence. J. Exp. Med., 201: 453-463.

Dalloul, R.A. and H.S. Lillehoj, 2006. Poultry coccidiosis: Recent advancements in control measures and vaccine development. Expert Rev. Vaccines, 5: 143-163.

Dubremetz, J.F., N. Garcia-Reguet, V. Conseil and M.N. Fourmaux, 1998. Apical organelles and host-cell invasion by Apicomplexa. Int. J. Parasitol., 28: 1007-1013.

El-Hajj, H., M. Lebrun, M.N. Fourmaux, H. Vial and J.F. Dubremetz, 2007. Inverted topology of the *Toxoplasma gondii* ROP5 rhoptry protein provides new insights into the association of the ROP2 protein family with the parasitophorous vacuole membrane. Cell Microbiol., 9: 54-64.

Hofmann, J. and W. Raether, 1990. Improved techniques for the *in vitro* cultivation of *Eimeria tenella* in primary chick kidney cells. Parasitol. Res., 76: 479-486.

Igonet, S., B.V.L. Normand, G. Faure, M.M. Riottot, C.H. Kocken, A.W. Thomas and G.A. Bentley, 2006. Cross-reactivity studies of an anti-*Plasmodium vivax* apical membrane antigen 1 monoclonal antibody: Binding and structural characterisation. J. Mol. Biol., 366: 1523-1537.

Lal, K., E. Bromley, R. Oakes, J.H. Prieto and S.J. Sanderson *et al*., 2009. Proteomic comparison of four *Eimeria tenella* life-cycle stages: Unsporulated oocyst, sporulated oocyst, sporozoite and second-generation merozoite. Proteomics, 9: 4566-4576.

Levine, N.D., 1988. Progress in taxonomy of the apicomplexan protozoa. J. Eukaryotic Microbiol., 35: 518-520.

Mercier, C., K.D. Adjogble, W. Daubener and M.F. Delauw, 2005. Dense granules: Are they key organelles to help understand the parasitophorous vacuole of all apicomplexa parasites? Int. J. Parasitol., 35: 829-849.

Nichols, B.A., M.L. Chiappino and G.R. O'Connor, 1983. Secretion from the rhoptries of *Toxoplasma gondii* during host-cell invasion. J. Ultrastruct. Res., 83: 85-98.

Proellocks, N.I., L.M. Kats, D.A. Sheffield, E. Hanssen, C.G. Black, K.L. Waller and R.L. Coppel, 2009. Characterisation of PfRON6, a *Plasmodium falciparum* rhoptry neck protein with a novel cysteine-rich domain. Int. J. Parasitol., 39: 683-692.

Richard, D., C.A. MacRaild, D.T. Riglar, J.A. Chan and M. Foley *et al*., 2010. Interaction between *Plasmodium falciparum* apical membrane antigen 1 and the rhoptry neck protein complex defines a key step in the erythrocyte invasion process of malaria parasites. J. Biol. Chem., 285: 14815-14822.

Shirley, M.W., A.L. Smith and F.M. Tomley, 2005. The biology of avian *Eimeria* with an emphasis on their control by vaccination. Adv. Parasitol., 60: 285-330.

Straub, K.W., E.D. Peng, B.E. Hajagos, J.S. Tyler and P.J. Bradley, 2011. The moving junction protein RON8 facilitates firm attachment and host cell invasion in *Toxoplasma gondii*. PLoS Pathog.

Straub, K.W., S.J. Cheng, C.S. Sohn and P.J. Bradley, 2009. Novel components of the Apicomplexan moving junction reveal conserved and coccidia-restricted elements. Cell Microbiol., 11: 590-603.

Xie, H.L., M.Q. Xie, H.X. Wu, X.Y. Peng, L.N. Wen and J.F. Zhang, 1994. Effects of media and their components on cell culture of Eimeria tenella. Poceedings of the 2nd Asian Conference on Coccidiosis, November 25-28, 1994, Guangzhou, China, pp: 99-1091.