Acquisition of exogenous haem is essential for tick reproduction

Perner Jan[1], Sobotka Roman[2], Šíma Radek[1], Konvičková Jitka[1], Sojka Daniel[1], Oliveira Pedro Lagerblad[3], Hajdušek Ondřej[1], Kopáček Petr[1]

[1] *Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice*
[2] *Institute of Microbiology, Czech Academy of Sciences, Třeboň*
[3] *Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular, Brasil, Brazil*

E-mail: perner@paru.cas.cz

Haem and iron homeostasis in most eukaryotic cells is based on a balancing flux between the opposing pathways of haem synthesis in a multi-enzymatic pathway and haem degradation mediated by haem oxygenase (HO). In our work, we show that haem and iron metabolism in ticks depart from its canonical functioning described for other eukaryotic organisms.

We showed, by thorough mining in available genome and transcriptome databases, that ticks possess an incomplete haem biosynthetic pathway. Our experiments confirmed that ticks do not synthesise haem *de novo*. Instead, ticks acquire haem from host haemoglobin and recycle it as a prosthetic group needed for their endogenous haemoproteins. Reduced levels of dietary haem/haemoglobin led to aborted larvae hatching suggesting a critical involvement of host haem in the embryogenesis of ticks. Furthermore, we have focused on the haem inter-tissue trafficking in ticks. Functional analysis by RNAi-mediated silencing revealed that haem transport and storage in ticks is facilitated by abundant large lipid transfer proteins. As serum supplementation with haemoglobin or myoglobin, rather than equimolar supplementation with haemin only, led to higher levels of haem deposits in tick eggs, we speculate that ticks express a specific receptor with affinity towards haemo-/myo-globin structure in the tick intestine.

We further demonstrated that ticks, as well as other mites, lack the gene encoding HO. Loss of HO seems to be an ancestral trait in evolution of mites, followed by loss of haem biosynthesis in the origin of ticks. We have experimentally shown that ticks, indeed, do not acquire iron from host haem/haemoglobin but rather from a host transferrin, a major non-haem iron transporter protein found in mammalian blood. The mode of internalisation of host transferrin in the tick intestine is not clear as a gene orthologue of *bona fide* transferrin receptor was not found in the tick genome.

Implementation of a novel tool of artificial membrane feeding allowed us, for the first time, to examine the nutritional requirements of ticks. Thus, we could show that haemoglobin is strictly required only as a source of haem but it can be fully substituted by plasma proteins in the provision of amino acids for vitellogenesis.

This work substantially extends the current knowledge of the haem auxotrophy in ticks and emphasises the importance of haem and iron metabolism as a target for anti-tick intervention.

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