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Identification of mechanisms involved in iron and haem uptake in *Bartonella birtlesii*: in silico and in vivo approaches

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INTRODUCTION

Bartonella birtlesii (*B. birtlesii*), like other *Bartonella* species, has to face various conditions with regard to the presence of haem in its environments (arthropods, mammals, intra- or extracellular). *B. birtlesii* has an intraerythrocytic lifestyle in its natural host, the mouse. Inside mice, the extracellular free haem concentration is low. In contrast, when invading and multiplying inside the erythrocyte, *B. birtlesii* has to challenge toxic high haem concentrations. Similar to other *Bartonella* species [1], the *B. birtlesii* genome does not encode for the haem biosynthesis pathway. The presence of a haem uptake system is thus important for *Bartonellae*. Here we investigated the function of two components of a putative haem uptake system encoded in *B. birtlesii*, and other *Bartonellae* genomes.

RESULTS AND DISCUSSION

In silico identification of *B. birtlesii* genes potentially involved in iron and/or haem uptake

Analysis of the *B. birtlesii* genome reveals that, similar to other *Bartonella* species, this α-proteobacterium contains only inner membrane iron ABC transporters. Genes encoding for an outer membrane iron transporter or a complete siderophore biosynthesis pathway are missing in the *B. birtlesii* genome. Also, the *B. birtlesii* genome does not encode for iron storage proteins (Ftn, Bfn, Dps). In contrast, *Bartonellae* genomes encode for a complete putative haem transport system (Fig. 1). Similar to other bacteria like *Neisseria meningitidis*, *Bartonellae* could use haem as an iron source after its transport and its degradation into the cytoplasm. Regulation of the iron-related processes in *B. birtlesii* could similarly to other α-proteobacteria involve Mur/Fur, RirA and Irr regulators encoded in its genome. Irr was shown to be involved in the regulation of an outer membrane haem transporter in *Bradyrhizobium japonicum*. The modulation of the level and the activity of the components of the haem uptake machinery might be important for the *Bartonellae* lifestyle. Characterisation of *Bartonella tribocorum hutA*, *tonB* and *exbB* mutants as abacteriaemic strains also underlines the importance of the haem uptake process in *Bartonellae* [2]. We undertook a functional characterisation of *B. birtlesii* HutA [3] and TonB [4], two components required for the transport of haem through the outer membrane. HutA, already characterised in *Vibrio cholerae*, transports haem through the outer membrane and requires energy transmitted by the TonB protein [3].

Expression and activity of TonB from *B. birtlesii* expressed in *Escherichia coli*

The *tonB* structural gene from *B. birtlesii* was amplified and cloned in vector pBAD24 giving pBAD24::*tonBB.bir*. To test activity of TonB from *B. birtlesii*, we introduced plasmid pBAD24::*tonBB.bir* in strain C600*tonB*. The strain C600*tonB* pBAD24::*tonBB.bir* was tested for growth on LB plates in the presence of the strong Fe^{2+} iron chelator dipyridyl (Dip). The *E. coli tonB* mutant that is impaired in the TonB-dependent iron Fe^{3+} transport systems cannot grow in the presence of

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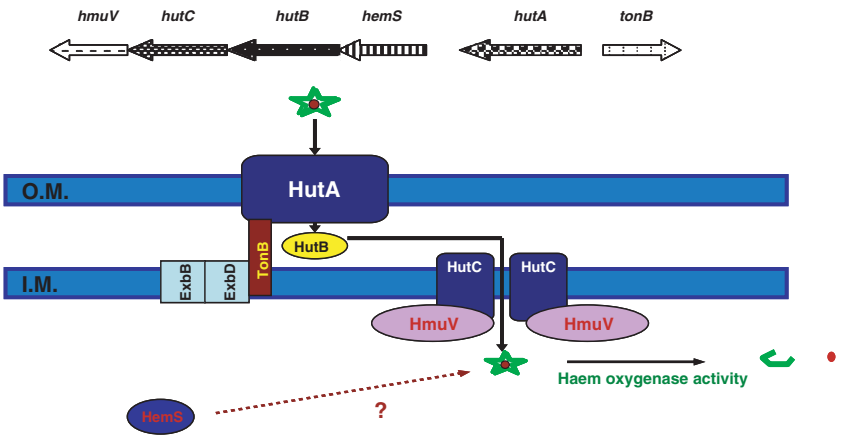

Fig. 1. The putative haem uptake system of *Bartonella birtlesii*.

Dip. Introduction of pBAD24::*tonBB.bir* plasmid into strain C600 *tonB* renders it able to grow in the presence of Dip. In contrast, the control strain C600*tonB* pBAD24 cannot grow in the presence of Dip. This result demonstrates that TonB from *B. birtlesii* can substitute for *E. coli* TonB to energise the TonB-dependent iron uptake system. Thus, TonB from *B. birtlesii* can interact with *E. coli* ExbB and ExbD, two other components of the energising complex TonB-ExbB-ExbD [4]. ExbB and ExbD are inner membrane proteins that can obtain energy from the proton motive force. This result is striking because the TonB protein from *B. birtlesii* is only 20% identical with the *E. coli* TonB, even in the C-terminus region that is responsible for the contact between TonB and the conserved TonB box of iron transporters [4]. For other TonB homologues, like TonB1 from *V. cholerae*, and HasB from *Serratia marcescens* [5], which are respectively 31% and 28% identical with TonB from *E. coli*, no energising activity toward *E. coli* iron uptake systems was put in evidence.

HutA from *B. birtlesii* cannot uptake haem in *E. coli* in the presence of its cognate TonB

As shown above, TonB from *B. birtlesii* can substitute TonB from *E. coli* for its energising activity. Specificity of HutA haem transporter towards a TonB protein was already demonstrated in *V. cholerae* [3]. We thus attempted to reconstitute the haem transportation activity of HutA from *B. birtlesii* in the presence of its cognate TonB. We introduced into strain C600 Δ*hemA* both plasmids pBAD24::*hutAB.bir* and pBAD24::*tonBB.bir*. The resulting strain was tested for growth in the presence of exogenous haemoglobin as haem source. Strain C600Δ*hemA* pBAD24::*hutAB.bir*, pBAD24::*tonBB.bir* cannot grow in the presence of haemoglobin added at 10^{-5} M. This result shows that, in spite of the presence of its cognate TonB, the HutA protein from *B. birtlesii* is not able to transport haem when expressed in *E. coli*. To explain this result, it can be hypothesised that other components of the outer membrane from *B. birtlesii* are required for the HutA haem uptake activity.

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