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Title

THE ROLE OF THE PARTNER SWITCHING MECHANISM IN REGULATION OF THE CHLAMYDIAL DEVELOPMENTAL CYCLE (http://opensiuc.lib.siu.edu/cgi/viewcontent.cgi?article=2032&context=dissertations)

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Abstract

Chlamydia spp. are obligate intracellular gram negative bacterial pathogens that cause infertility, blindness, and pneumonia in humans. The unique developmental cycle of Chlamydia spp. requires these bacteria to possess a mechanism(s) to control differentiation between two forms: the elementary body (EB) and the reticulate body (RB). Although the transition between these two phases is essential for the chlamydial infectious cycle (making the steps ideal drug targets), the signals triggering and the mechanisms carrying out differentiation are unclear. We hypothesize that the pan-chlamydial partner switching mechanism (PSM) is involved in RB to EB morphogenesis through the conversion of external signals into phenotypic changes via regulation of sigma28. The putative chlamydial PSM is composed of two sensor phosphatases (SPs: RsbU and CTL0852), two anti-anti-sigma factors (AASFs: RsbV1 and RsbV2), an anti-sigma factor (ASF: RsbW), and sigma28. Using the Bacterial Adenylate Cyclase Two Hybrid System (BACTH) and alternative protein protein interaction methods, we mapped the PSM interactome. Results revealed interactions between RsbU and both RsbV1 and RsbV2. Interestingly, while AASF partners for CTL0852 were not identified, interactions between CTL0852 and RsbU were observed indicating that CTL0852 may have a regulatory role for the phosphatase function assigned to RsbU. Our results also showed that the interaction between RsbV1 and RsbW was stronger than interactions between RsbV2 and RsbW. Further characterization of these interactions revealed that RsbV1 and RsbV2 compete to regulate RsbW and the AASFs and SF can form homo and hetero dimers. rsbW transcripts were detected 4-48 hours post infection, while rsbV2 transcripts were detectable 8-48 hours post infection. In contrast, rsbV1 and ctl0852 transcripts were found 24-48 hours post infection, while rsbU transcripts were detectable at 12, 24, 30 hours post infection. Our findings suggest that RsbW is present early during infection to bind sigma28 and prevent premature transcription of late genes. Late during infection (prior to RB to EB transition), ctl0852, rsbU, and rsbV1 are transcribed. CTL0852 may bind and regulate the activity of RsbU (potentially during stress responses), while RsbU may sense a signal(s) during normal growth stimulating it to dephosphorylate RsbV1 and RsbV2 leading to the dissociation of RsbW from sigma28. Sigma28 would then be free to initiate transcription of late genes needed for RB to EB conversion. To the best of our knowledge, this is the first report detailing the top half of the PSM interactome. In addition, we demonstrated competition between the chlamydial AASFs for binding the ASF and revealed dimerization of the ASF and AASFs. Finally, we tested the timing of PSM component transcription during normal infection. Our results will allow future studies to: 1) search for signals controlling the PSM system, 2) define its exact role in development, and 3) screen for inhibitors.