5 Final discussion

The work presented in this thesis represents an attempt to define the *C. difficile spo0A* regulon, and to underpin its role in disease and transmission. We took a two-step approach to this end. Firstly, we exploited a murine model of infection that mimics several aspects of *C. difficile* disease, persistence and transmission in humans, thus enabling us to assess the role of the *spo0A* gene in *C. difficile* pathogenicity. Secondly, we adopted an integrated transcriptomic and proteomic approach in order to provide a molecular description of the *C. difficile* Spo0A regulon at the whole genome level, and link this back to any role in disease and transmission.

Data from this study have demonstrated that the *C. difficile spo0A* gene is a highly pleiotropic global transcriptional regulator that coordinates multiple virulence, sporulation and metabolic phenotypes during *C. difficile* disease and transmission. A summary of the phenotypes under Spo0A regulation is given in Figure 5.1.

5.1 A murine model of *C. difficile* infection: insights into the role of Spo0A in persistence and transmission

The availability of a murine infection model opens a tractable route to study the genetic basis of *C. difficile* disease, relapse, persistence and transmission. In the work described in Chapter 3, we demonstrate for the first time that the *spo0A* gene of clinically relevant *C. difficile* ribotypes is essential for persistent infection and efficient host-to-host transmission in mice. Importantly, we also show that a functional *spo0A* gene is required for relapsing infection

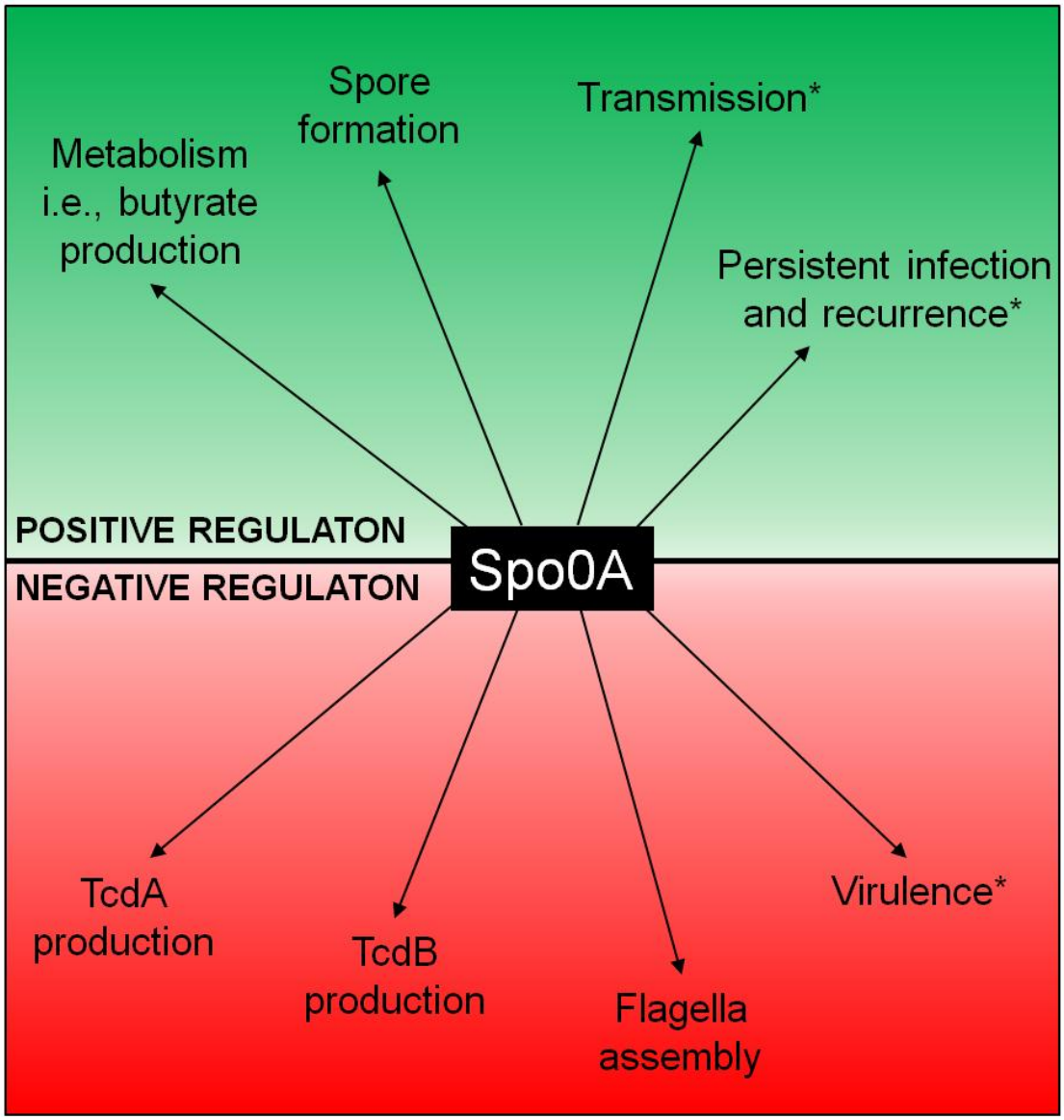

Figure 5.1. Summary of the phenotypes associated with the *C. difficile* Spo0A regulon. *C. difficile* Spo0A is a highly pleiotropic global transcriptional regulator that coordinates multiple phenotypes associated with metabolism, disease, persistence and transmission in mice. Phenotypes were identified using both the mouse model of *C. difficile* infection and transcriptomic and proteomic approaches. *According to our murine model of *C. difficile* infection.

after vancomycin therapy, and we further illustrate that the local environment may serve as a reservoir of *C. difficile* infection. These observations have potential practical implications relating to the management of hospital patients.

Additionally, we provide evidence that *C. difficile* Spo0A mutant derivatives produce higher levels of the toxins TcdA and TcdB *in vitro* compared to the parental equivalents, which was associated with enhanced virulence *in vivo*. Thus, Spo0A may negatively regulate *C. difficile* toxin production. Although this finding is potentially contradictory to the observations of Underwood *et al*. (2009), we believe that our approach was comparatively more robust and quantitative. Additionally, our observed phenotype was supported by the transcriptional data described in Chapter 4.

5.2 Insights from an "omics" approach to studying the *C. difficile* Spo0A regulon

The work described in Chapter 4 exploits a combined transcriptomic and proteomic approach to define the *C. difficile spo0A* regulon at the whole genome level. Such an integrated approach allowed us to derive a holistic picture of *C. difficile* biology that could not have been readily achieved using each approach independently and identified multiple novel phenotypes associated with the Spo0A regulon. We have determined that Spo0A is a negative regulator of *C. difficile* flagellar assembly and a positive regulator of several metabolic pathways, including the fermentation of carbohydrates leading to the production of the SCFA butyrate.

Importantly, we have also validated Spo0A as a transcriptional regulator of a number of sporulation genes, and confirm that Spo0A negatively regulates toxin production in *C. difficile* 630Δ*erm* at the transcriptional level.

5.3 Final conclusions and future directions

C. difficile is an enteropathogen that in the past two decades has emerged from relative obscurity to become a dominant healthcare-associated pathogen. Human-virulent ribotypes continue to disseminate globally and appear to be associated with increased incidence and severity, representing a major medical burden and significant economic drain on finite resources.

In the present study, we have demonstrated that Spo0A regulates multiple *C. difficile* phenotypes associated with disease, persistence and transmission in mice. However, many questions still remain and should inform the direction of future research. For example, due to the complex pleiotropy of the *spo0A* mutation, we were unable to provide a full molecular description of the events that occur during the course of spore formation in *C. difficile*. Work to generate and characterise a *C. difficile sigE* mutant is currently in progress. Such a mutation should also generate an asporogenous phenotype, but *sigE* is active at a later stage in the sporulation cascade and thus the number of pleiotropic effects may be reduced compared to *spo0A*. In addition, we do not at present have data on the repertoire of proteins secreted by

C. difficile. This information may prove useful in identifying novel secreted virulence-associated factors or mediators of host – pathogen interactions.

During this study, we identified novel phenotypes associated with the *C. difficile* Spo0A regulon. For example, we demonstrated that Spo0A negatively regulates several components of the *C. difficile* flagellar assembly apparatus. In addition, we also established Spo0A as a positive regulator of several metabolic pathways, including the fermentation of carbohydrates leading to the production of the SCFA, butyrate. These phenotypes have potential implications relating to the persistence and colonisation of *C. difficile in vivo* (discussed in chapter 4.4). As such, work is currently underway to generate defined insertional mutations in the *fliC* and *fliD* (flagella) genes, as well as the *thlA1* and *hbd* (butyrate biosysthesis) genes of both *C. difficile* R20291 and *C. difficile* 630Δ*erm*. The role of such genes in *C. difficile* disease, persistence and colonisation can then be determined using our mouse model of infection.