6 Final Discussion

IFITM3 is a potent anti-viral restriction factor that protects cells against infection by viruses from 10 different families, including influenza virus, dengue virus and West Nile virus[1,2]. This has been shown both *in vitro* and for some viruses in a knock-out *Ifitm3* $^{-/-}$ mouse model[3]. IFITM3 is localised to late endosomes and prevents fusion of the virus and host membranes. The prevention of viral pore formation precludes the release of viral nucleic acids into the cytoplasm[4-6].

In this thesis the *IFITM3* gene of patients infected during the H1N1 pandemic in 2009 were sequenced and the prevalence of SNPs in this locus compared to ethnically-matched controls. In particular, the rare C allele of SNP rs12252 was identified as being over-represented in the genomes of hospitalised patients. This association between the C allele at SNP rs12252 and severe influenza symptoms has since been replicated by two further studies[247,248].

The function of the allele at rs12252 was subsequently investigated. Automated annotation of this locus in Ensembl suggested that alternative *IFITM3* transcripts may be transcribed from a different promoter, potentially creating an N-terminally truncated protein. We hypothesised that the recessive C allele would increase the abundance of truncated proteins with respect to the full-length proteins, explaining the poor response to influenza shown by these patients. Using quantitative RT-PCR, we detected the transcription of alternative transcripts in both primary airway epithelial cells and lymphoblastoid cells. However, due to a lack of primary cells with different ethnicities and antibodies that could distinguish between IFITM2 and IFITM3, no association was found between the rs12252 allele and the ratio of the transcripts in these cells. Furthermore, an N-terminally truncated form of IFITM3 has not been detected *in vitro.* In order to investigate the function of the allele at rs12252 more thoroughly, reagents with higher specificity are required. Additionally, re-sequencing of the IFITM locus is needed to allow the identification of SNPs that are in linkage disequilibrium (LD) with rs12252, which may be having an effect on IFITM3 expression. Currently, the gaps in this region prevent this analysis from being carried out (Figure 66).

IFITM3 was shown to be an important restriction factor in other mammals, such as mice and marsupials, but this locus had been neglected in birds due to poor

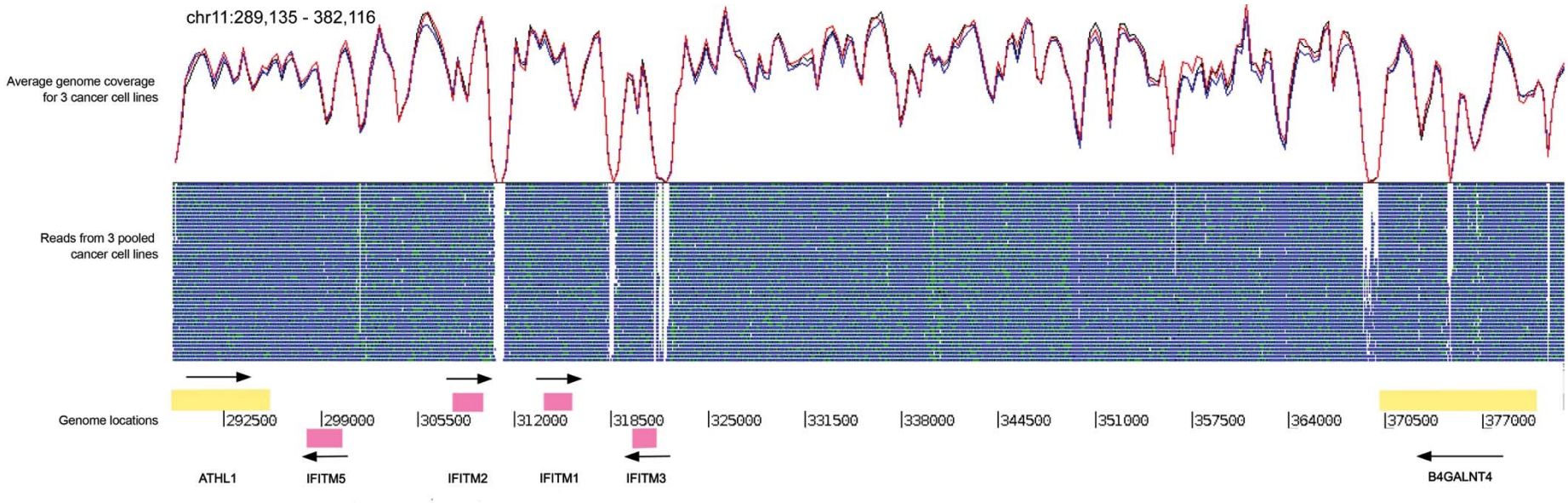

Figure 67: Low coverage regions in IFITM locus

Stacked Illumina sequencing reads are shown for three cancer cell lines (blue) for the *IFITM* locus (289,135 – 382,116), uncovered regions are shown in white. The average genome coverage for each cell line is shown as a continuous line. Flanking genes *ATHL1* (289,135-296,107) and *B4GALNT4* (369,804-382,116) are shown in yellow, and *IFITM* genes (*IFITM5* [298,200-299,526]; *IFITM2* [308,163-309,395]; *IFITM1* [313,853-313,272]; *IFITM3* [319,669-321,050]) are shown in pink. Genome locations are in brackets.

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sequence coverage. Wild fowl are an important reservoir for influenza infection, and chickens are particularly susceptible to highly pathogenic strains, such as H5N1. In this thesis the human *IFITM3* transcript was used to perform BLAST searches on the chicken genome and identified three orthologous IFITM proteins. These proteins were over-expressed in human lung epithelial cells (A549s) and were shown to restrict several HA subtypes of influenza virus and two lyssaviruses. Since chickens are not normally infected by lyssaviruses this showed that the mechanism of anti-viral activity is likely to be non-specific and therefore based on a generic process which all enveloped viruses carry out. Further investigation showed that DF-1 chicken cells expressed endogenous *IFITM3,* and that siRNA knock-down of this gene resulted in an increase in influenza A replication. The contrary was also true – over-expression of chIFITM3 resulted in a decrease in IAV infection in DF-1 cells. Understanding the diversity of this locus in different chicken breeds is important for identifying which chicken lines important to the poultry industry are more or less susceptible to IAV infection.

In order to understand the mechanism employed by IFITM3 to restrict viral entry a number of cell based signalling assays were carried out. However IFITM3 was not shown to signal via an ISRE, an IFNβ promoter or NF-κB. TLR stimulation using synthetic agonists had no impact on signalling either. Infection by influenza A WSN/33 post-transfection reduced the signalling stimulated by all positive controls (MAVS and tetherin) as well as mutant tetherin, IFITM3 and the empty control, so it is unlikely this was an IFITM3-specific effect. Furthermore a co-IP was optimised to pull down the interacting partners of IFITM3 under different conditions. In future this could be used in combination with mass spectrometry to identify all proteins, not just hypothesised interactions.