Abstract

The aim of the project was to define the function of the African swine fever virus (ASFV)-encoded ubiquitin conjugating enzyme (UBCv1). Two alternative approaches were taken to construct recombinant ASFV in which either (i) a functional UBCv1 was not expressed or (ii) the UBCv1 gene was controlled by an inducible promoter so that its expression could be regulated. It was anticipated that the regulated gene approach would produce viable recombinant viruses even if the UBCv1 gene was essential for infection.

First, a replacement plasmid was made to delete the wild-type gene from the ASFV genome. Then, an inducible ASFV promoter containing the lac operator was cloned upstream of the UBCv1 gene and expression of UBCv1 was shown to be regulated by IPTG when co-transfected in infected cells with another plasmid expressing the lac repressor. Transfer plasmids were constructed to recombine this inducible UBCv1 gene into either the wild-type UBCv1 locus or, as a second copy into a non-essential locus in the genome. None of these approaches produced viable recombinant viruses, suggesting that UBCv1 is an essential gene whose level and timing of expression are important for the viability of ASFV.

To identify possible substrates for the UBCv1, the gene was used as bait to screen a pig macrophage cDNA library using the yeast two-hybrid system. Six clones encoding proteins which interacted specifically with the UBCv1 protein were isolated. Sequencing of the inserts in these clones showed that three encoded ubiquitin. This was expected since adenylated ubiquitin is a common substrate for all UBC enzymes with which they interact as they transfer the ubiquitin to substrate proteins. One interesting UBCv1-interacting protein was contained in a clone encoding part of a protein named SMCp which was very similar to the N-terminal region of the mammalian SMC and retinoblastoma binding protein-2 (RBP2) genes. The RBP2 protein is thought to have an important role in regulating cell division by regulating the function of the retinoblastoma protein. *In vitro* binding studies confirmed that UBCv1 binds GST-SMCp but does not bind GST. Immunofluorescence studies showed that UBCv1 was present in the nucleus in some cells and cytoplasm in others suggesting it shuttles between the nucleus and the cytoplasm. The specific interaction of UBCv1 with SMCp and the subcellular localisation of UBCv1 suggest that SMCp may be a substrate *in vivo* for the enzyme. The possible significance of this is discussed.