An ARID family protein binds to the African swine fever virus encoded ubiquitin conjugating enzyme, UBCv1

Wallace D. Bulimo, James E. Miskin, Linda K. Dixon*

Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Surrey GU24 0NF, UK

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Abstract The NH_2-terminal end of a protein, named SMCp, which contains an ARID (A/T rich interaction domain) DNA binding domain and is similar to the mammalian SMCY/SMCX proteins and retinoblastoma binding protein 2, was shown to bind the African swine fever virus encoded ubiquitin conjugating enzyme (UBCv1) using the yeast two hybrid system and in in vitro binding assays. Antisera raised against the SMCp protein were used to show that the protein is present in the cell nucleus. Immunofluorescence showed that although UBCv1 is present in the nucleus in most cells, in some cells it is in the cytoplasm, suggesting that it shuttles between the nucleus and cytoplasm. The interaction and co-localisation of UBCv1 with SMCp suggest that SMCp may be a substrate in vivo for the enzyme.

Key words: Ubiquitin conjugating enzyme; African swine fever virus; A/T rich interaction domain; Ubiquitin; SMCX

1. Introduction

African swine fever virus (ASFV) is a large DNA virus which replicates in the cytoplasm of infected cells and has a tropism for macrophages. The 170 kb genome [8,32] encodes the only known virus encoded ubiquitin conjugating enzyme, UBCv1 [15,27].

These enzymes catalyse the attachment of ubiquitin to substrate proteins which tags them for proteolytic degradation via the proteasome. In addition to UBC enzymes, ubiquitin activating and E3 or ubiquitin protein ligase enzymes (for review see [6,14,28,29]) are involved in attaching ubiquitin to substrate proteins. Selective protein degradation mediated by protein ubiquitination regulates many processes including transcription and cell cycle control [6,26,29].

Recombinant UBCv1 can, in vitro, ubiquitinate itself as well as histones and an ASFV virion protein, PIG1 [15,16]. However, it is not known whether these are in vivo substrate(s) for the enzyme. To investigate putative substrates for UBCv1, we identified interacting host proteins using the yeast two hybrid system. Among the UBCv1 interacting proteins was a truncated protein containing 215 amino acids including an ARID (A/T rich interaction domain) DNA binding domain. This protein, named SMCp, is very similar to the SMCX/Y [1,31] and retinoblastoma binding protein 2 (RBBP2) [10]. We demonstrated specific binding in vitro of UBCv1 to this 215 amino acid domain of SMCp and showed that both proteins are predominantly localised in the nucleus in cells. These results suggest either that SMCp is an in vivo substrate for UBCv1 or that the interaction targets UBCv1 to another substrate.

2. Materials and methods

2.1. Cells and viruses

Primary lamb testis (LT), bovine kidney (BK) cells, IBRS2 (ECACC 84100503), RK-13 (ECACC 88062427), HeLa (ECACC 84121901), CHO-K1 (ECACC 85051005), Vero (ECACC 84113001) and BSC 1 (ECACC 85011422) cells were grown under 5% CO_2 at 37°C in HEPES buffered Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum. BA71V tissue culture adapted ASFV isolate has been described previously [4].

2.2. Plasmids

A *Sma*I, *Sal*I fragment containing the *UBCv1* gene was cloned into pAS2-CYH2 (Clontech) as an in frame fusion with the GAL4 DNA binding domain. The insert in the plasmid was sequenced to confirm that the *UBCv1* ORF was in frame with the GAL4 DNA binding domain.

SMCp was amplified by PCR from the pACT2.6 AD plasmid, using primers SMCp-FOR; AAA GGA TCC ATG GAG CCG GGG TCA GAC G and SMCp-REV; AAA GAA TTC GGG TTT TTC AGT ATC TAC GAT TC. The amplified fragment of 650 bp was digested with the *Bam*HI and *Eco*RI and ligated into pGEX2TK vector (Pharmacia-Amersham) to give the pGEX2TK-SMCp plasmid.

The *UBCv1* gene was cloned as a *Bam*HI fragment downstream and in frame with an eight amino acid epitope tag derived from human influenza virus haemagglutinin (HA) protein to give pCDNA-HA-UBCv1.

The HA tagged UBCv1 gene cassette was released from pCDNA-HA-UBCv1 using *Hin*dIII and *Xba*I, end repaired and ligated to *Sma*I digested p32-OP plasmid (L.K. Dixon and C.C. Abrams, unpublished results), downstream of the ASFV early/late p32 promoter [25] in the pT7 plasmid (Invitrogen), to give the plasmid p32-OP-HA-UBCv1.

Primers NLS Mut1 (5′-GAT CTA CTA GTC ATA TGG ATT GG-3′) and NLS Mut2 (5′-ATT CTA GAT CCT CTT TAT ATA GAT ATT TAC GGT AG-3′) were used to amplify 700 bp containing the p32 promoter, the HA epitope and the 5′ portion of UBCv1 upstream of the putative NLS. Primers NLS Mut3 (5′-GAG GAT CTA GAA TCA TAC CCC ATG GAA GAG TGT TCA GCG GAA GAC ATA G-3′) and NLS Mut4 (5′-GTG TGA CTA TAG CTA CTC ATC ATC CAT CTC TT-3′) amplified 200 bp of the 3′ terminus of UBCv1 after the putative NLS. The 700 bp and 200 bp PCR fragments were digested at *Xba*I sites within the primers, ligated together and then cloned into pT7Blue2 to give p32-OP-HA-UBCv1 (NLS Mut). The 700 bp 5′ fragment was also cloned separately into pCDNA3 giving p32-OP-HA-UBCv1 ΔC.

2.3. Yeast two hybrid screen

pAS2-UBCv1 plasmid was co-transformed with a porcine macrophage cDNA library [23] into yeast strain Y190. Colonies containing plasmids encoding UBCv1 interacting proteins were selected by growing on medium lacking histidine in the presence of 25 mM 3-aminotriazole. Specificity of the interaction with UBCv1 was confirmed by retransformation into yeast with either the pAS2-UBCv1 plasmid or other control plasmids and testing for expression of the Gal 4 dependent reporter β-galactosidase. Inserts in plasmids were sequenced and sequences compared with sequence databases using BLAST [2,3].

*Corresponding author. Fax: (44)-1483-232448.
E-mail: linda.dixon@bbsrc.ac.uk

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2.4. Immunofluorescence

Vero cells were grown on coverslips, infected with BA71V virus and transfected with 10 μg of the appropriate plasmid. After overnight incubation coverslips were fixed in 3% paraformaldehyde and permeabilised with 0.2% (w/v) Triton X-100. After washing, cells were blocked in 0.2% gelatin in phosphate buffered saline at room temperature and incubated with rat anti-HA primary antibody (1/100) followed by FITC conjugated secondary goat anti-rat antibody (1/50), then mounted in Mowiol (Hoechst) and examined using a fluorescence microscope.

2.5. RT-PCR amplification of SMCp and SMCp-like genes

Total RNA from cell lines or pig tissues was isolated using the Qiagen total RNA extraction kit. RT-PCR was carried out using primers SMCp-FOR and SMCp-REV.

2.6. Expression of GST and GST fusion proteins

Expression of the GST or GST–SMCp fusion proteins was induced in *Escherichia coli* transformed with either pGEX2TK or pGEX2TK-SMCp plasmids by adding IPTG to 0.1 mM. Proteins extracted from bacteria were affinity purified using glutathione Sepharose beads (Pharmacia-Amersham) and analysed by SDS–PAGE followed by staining with Coomassie brilliant blue.

2.7. Binding of HA-UBCv1 to SMCp

Radiolabelled HA-UBCv1 was produced by in vitro transcription and translation from plasmid pCDNA-HA-UBCv1 (Promega TNT). Bacterially expressed GST and GST–SMCp fusion protein were bound to glutathione Sepharose beads (Pharmacia-Amersham) and incubated at 4°C overnight with radiolabelled in vitro translated HA-UBCv1 at varying salt concentrations (50–200 mM NaCl) in 50 mM Tris–HCl pH 7.5, 0.1% (v/v) NP40. The beads were washed three times in the binding buffer and bound proteins separated by SDS–PAGE and visualised by fluorography.

2.8. Production of antibodies against SMCp

75 μg of purified GST–SMCp protein was inoculated into a rabbit at 2 week intervals both intramuscularly and subcutaneously in Freund's complete adjuvant. Serum was collected at day 54 post inoculation and anti-SMCp antibodies were purified by passing through a column containing GST coupled to Sepharose 4B beads (Pharmacia-Amersham). The flowthrough was loaded onto a column containing GST–SMCp coupled to Sepharose 4B beads and bound SMCp specific antibodies were eluted with 100 mM glycine–HCl, pH 2.5 buffer.

3. Results

3.1. Identification of pig proteins which bind UBCv1

To identify cellular proteins that may be substrates for UBCv1, we looked for proteins that interact with this enzyme by screening a pig alveolar macrophage cDNA library [23] using the yeast two hybrid system [5,9]. A plasmid vector (pAS2-UBCv1) that expressed UBCv1 protein as a fusion with the DNA binding domain of GAL4 was used as bait.

Four colonies containing plasmids that expressed UBCv1 interacting proteins were isolated. Plasmids were isolated from these clones and retransformed into yeast together with the pAS2-UBCv1 plasmid or an irrelevant GAL4 DNA binding domain fusion (pGBT9-A238L) and assayed for expression of β-galactosidase (β-gal; Table 1). This confirmed that the interaction between UBCv1 and the proteins encoded by the four library clones was specific. DNA inserts from the library clones were sequenced and searched against DNA databases using BLAST [2,3]. Three of the four positive clones contained cDNAs encoding ubiquitin and shared 100% amino acid identity at the protein level with porcine and human ubiquitin. Each of these also contained nucleotide sequences downstream of the ubiquitin open reading frame showing that they were ubiquitin fusions with other genes. This is expected because ubiquitin is always expressed either as polyubiquitin or fused with other proteins [12,20,21,24,30].

One of the clones encoding a UBCv1 interacting protein (pACT2.6) contained a truncated part of a gene encoding a protein we designated SMCp. Within a 581 bp overlap, this *SMCp* gene shared 93% nucleotide identity with the 5′ region of the gene encoding human SMCX protein. At the protein level, the *SMCp* encoded protein shared 94% amino acid identity with human SMCX and 88% with the human SMCY proteins over the NH_2-terminal 201 amino acids (Fig. 1), 69% identity with human RBBP2 over 168 amino acids and 47% identity with the mouse Jumonji protein over 156 amino acids. SMCX, SMCY, RBBP2 and Jumonji contain ARID DNA binding domains which are involved in eukaryotic gene regulation [13]. The ARID domain is contained within the region of the SMCp protein encoded by pACT2.6.

3.2. UBCv1 binds to SMCp in vitro

To confirm the interaction observed between UBCv1 and SMCp we carried out binding assays using in vitro translated HA-UBCv1 and recombinant GST–SMCp proteins. Expression of the GST–SMCp protein (54 kDa) was induced by addition of IPTG to *E. coli* cultures containing the pGEXTK-SMCp plasmid. The UBCv1 gene fused downstream of a sequence encoding the HA epitope tag under the control of the T7 promoter in the pCDNA3 vector (pCDNA3-HA-UBCv1) was transcribed and translated in vitro to produce radiolabelled HA-UBCv1 (Fig. 2, lane 7). The GST–SMCp fusion protein and a control GST protein were bound to glutathione Sepharose beads and incubated overnight at 4°C with [^{35}S]methionine labelled HA-UBCv1 in binding buffer containing varying concentrations of NaCl (50, 100 and 200 mM). The beads were collected and washed in binding buffer, then proteins were separated by SDS–

Table 1
Yeast two hybrid screen of pig macrophage cDNA library with UBCv1 gene

Bd plasmid	Ad plasmid	β-Gal expression	Database matches	Nucleotide identity (%)
pAS2-UBCv1	none	no		
pAS2-UBCv1	pACT2-A238L	no		
pAS2-UBCv1	pACT2.5	yes	*H. sapiens* Uba80 mRNA for ubiquitin; X63237	466/503 = 92%
pAS2-UBCv1	pACT2.6	yes	*H. sapiens* XE169mRNA; L25270	544/581 = 93%
pAS2-UBCv1	pACT2.46	yes	*H. sapiens* Uba80 mRNA for ubiquitin X63237	442/478 = 92%
pAS2-UBCv1	pACT2.R	yes	pig poly-ubiquitin mRNA M18159	483/483 = 100%

The plasmids used as the Gal4 DNA binding domains (Bd plasmid) or activation domains (Ad plasmid) are indicated. Activation of the Gal 4 dependent β-galactosidase gene was assayed using a colony lift assay [9]. Sequences of inserts from clones isolated from a pig macrophage cDNA library encoding proteins that interact with UBCv1 were compared with databases and those entries showing the highest similarity are indicated together with the % nucleotide identity and length of sequence over which this identity is measured.

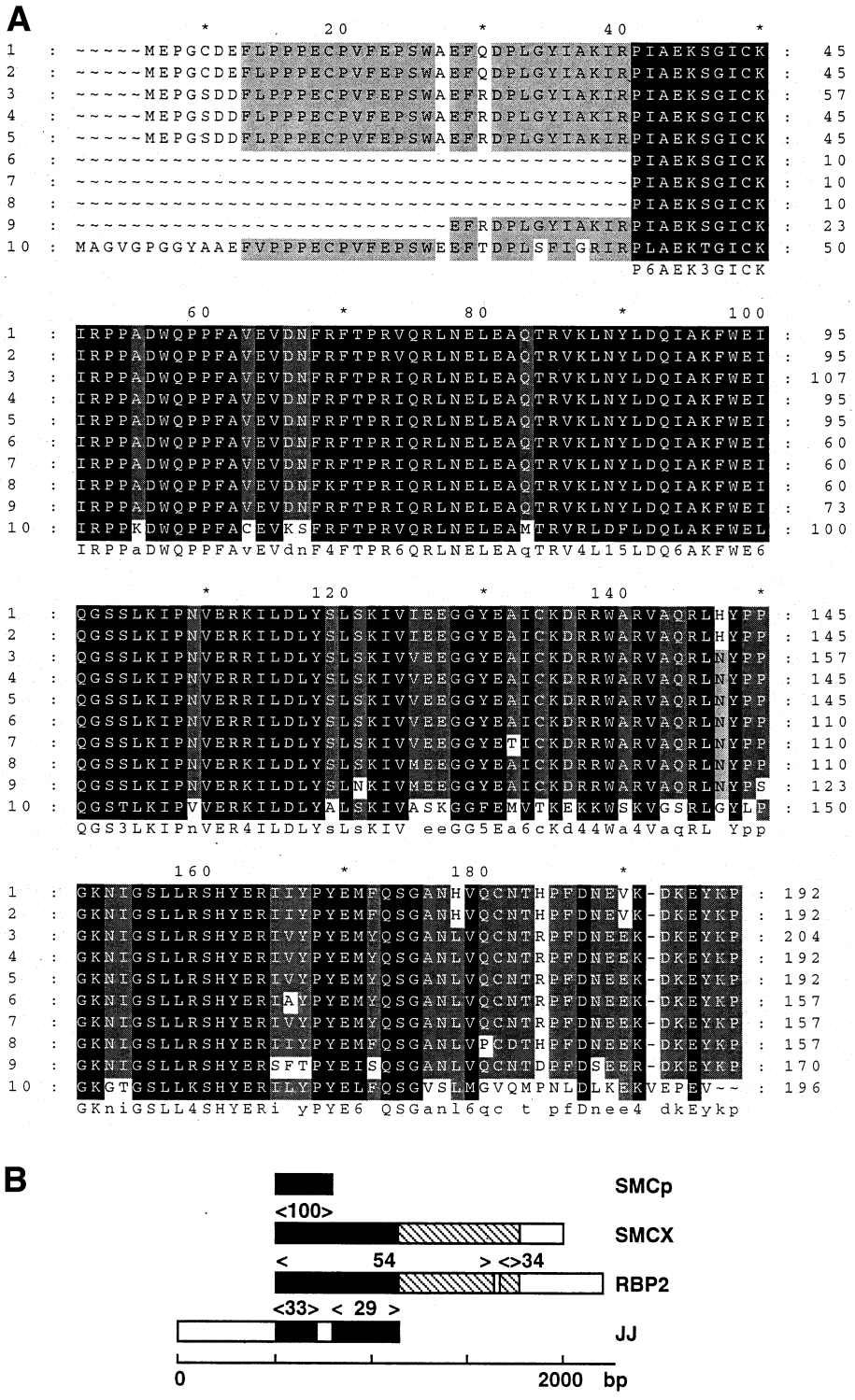

Fig. 1. Alignment of SMCp protein with related SMCX/Y and RBBP2 proteins. A: Alignment of the SMCp protein sequence (5) with similar SMCX/Y and RBBP2 proteins. The GCG pretty box program was used to align the sequences. Sequences shown are human SMCY protein (1), human SMCY (H-Y) protein (2), human XE169 protein (3), human SMCX protein (4), *Mus domesticus* SMCX protein (6), *Equus caballus* SMCX protein (7), *Equus caballus* SMCY protein (8), *Mus musculus* (BALB/c) SMCY protein (9) and human retinoblastoma binding protein 2 (10). Black shading shows identical residues; grey shading indicates similar residues. The last line of sequences corresponds to a computed consensus sequence. B: Conserved domain in SMCX, RBBP2 and Jumonji (JJ) proteins. Regions conserved between the SMCX/Y, RBP2 and Jumonji proteins are shown in black. Regions of similarity between SMCX/Y and RBP2 but not Jumonji proteins are shown in grey. The percentage amino acid identity between two adjacent protein sequences in the alignment is indicated.

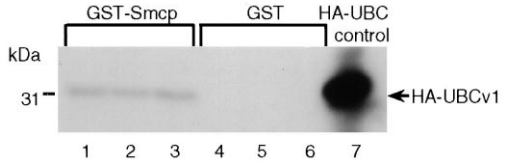

Fig. 2. In vitro translated HA-UBCv1 binds to GST-SMCp but not to GST proteins. Bacterially expressed GST–SMCp and GST proteins were bound to glutathione Sepharose beads and incubated with in vitro transcribed and in vitro translated radiolabelled HA-UBCv1 in the presence of SMCp binding buffer (see Section 2) containing 50 mM (lanes 1 and 4), 100 mM (lanes 2 and 5), or 200 mM (lanes 3 and 6) NaCl and 0.1% NP40. Beads were washed three times with the binding buffer and bound proteins eluted with SDS–PAGE sample buffer, then separated by 10% SDS–PAGE. Bound HA-UBCv1 was visualised by fluorography after separation of the proteins using SDS–PAGE.

PAGE and radiolabelled proteins detected by fluorography. Binding of HA-UBCv1 to GST–SMCp was detected at NaCl concentrations between 50 and 200 mM (Fig. 2, lanes 1–3) but no binding to the control GST protein was detected (Fig. 2, lanes 4–6). These results confirm that UBCv1 binds specifically to the 192 N-terminal domain of SMCp. Due to the very large size of SMCX/SMCY and RBP2 binding proteins (>1500 amino acids), expression of the full length protein would be technically very difficult to achieve and may not produce correctly folded protein. The 215 amino acid region of the protein we used in binding studies contains the intact ARID DNA binding domain which lies between residues 76 and 184. To fulfill its function, this region of the protein is likely to be exposed on the surface of the intact SMCp protein. Moreover, DNA binding domains from transcription regulators have been shown to retain their function when expressed separately from the rest of the protein or as fusions with other proteins [11,18]. Thus we expect this part of the protein to be correctly folded when expressed as a separate domain. The specific binding we observed between UBCv1 and the truncated N-terminal domain of SMCp is therefore likely to be true for the intact SMCp protein.

3.3. Transcripts of SMCp are present in a wide range of tissues and cell lines

The presence of SMCp transcripts in various pig tissues was studied by RT-PCR. A band of the expected size (650 bp) was amplified from RNA from ovary, brain, liver, spleen, skeletal and cardiac muscle whereas no bands were amplified in control experiments (Fig. 3). The human *SMCX* gene is similarly expressed in a wide variety of tissues [31].

To determine if the *SMCp* gene is conserved in different species, its expression in a variety of mammalian primary and differentiated cells was studied by RT-PCR. The expected 650 bp fragment was amplified from Vero (monkey kidney), BSC1 (monkey kidney), HeLa (human epithelial), RK13 (rabbit kidney), IBRS2 (pig kidney), LT (lamb testis), BK (bovine kidney) and CHO-K1 (Chinese hamster ovary) cells (Fig. 3). Previous studies have shown that *SMC* genes are expressed in many tissues of all mammalian species including wallaby and *Monodelphis domestica* (metatherians), human, horse, dog, mouse, cattle, sheep and rabbits (eutherians [19]). These observations, combined with the high sequence similarity, support the suggestion that SMCp is a porcine homologue of SMCX/Y proteins.

3.4. SMCp localises to the nucleus of ASFV infected cells

We studied subcellular localisation of SMCp and UBCv1 to determine if the proteins could interact in ASFV infected cells. A rabbit was immunised with bacterially expressed GST–SMCp and anti-SMCp specific antibodies were purified by affinity chromatography. Western blotting confirmed that this purified antibody specifically recognised the bacterially expressed SMCp protein cleaved from the GST–SMCp fusion (data not shown). This antibody was used to determine the subcellular localisation of SMCp in uninfected and ASFV infected Vero cells using indirect immunofluorescence. This showed that SMCp localised to the nucleus of all cells (Fig. 4d). No staining was observed using non-immune rabbit sera, nor with either primary or secondary antibody alone (data not shown).

3.5. UBCv1 localises mainly to the nuclei of ASFV infected cells

To study the subcellular localisation of UBCv1 in ASFV infected cells, a plasmid with the HA epitope fused to the *UBCv1* gene under the control of the strong early/late p32 ASFV promoter was transfected into Vero cells infected with ASFV BA71V isolate. The HA tagged UBCv1 enzyme was detected using a rat anti-HA monoclonal antibody and FITC conjugated goat anti-rat secondary antibody. In a small proportion of the cells staining was localised to the cytoplasm alone and in a few cells both the cytoplasm and the nucleus were stained (Fig. 4a,b). However, the majority of the cells displayed staining localised to the nucleus alone (Fig. 4c). No staining was observed in cells reacted with primary or secondary antibody alone nor in cells which were infected but not transfected (data not shown). These observations suggested that HA-UBCv1 shuttles between the cytoplasm and the nucleus of ASFV infected cells but is mainly located in the nucleus.

UBCv1 contains the amino acid sequence, VKKTVKKSLD, towards its carboxy-terminus, residues 161–170, which is similar to the nuclear localisation signal (NLS) of human c-myc protein [22]. To test whether this sequence is needed for the transport of UBCv1 into the nucleus, the sequence encoding these residues was deleted from UBCv1. This plasmid

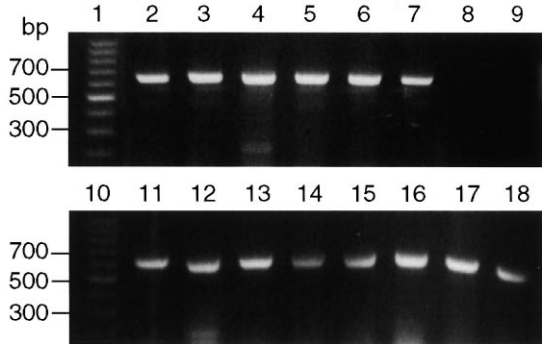

Fig. 3. SMCp is transcribed in a wide variety of tissues and cell lines. Total RNA was prepared from pig tissues or cell lines and RT PCR carried out using primers specific for the SMCp gene. Lanes 1 and 10 show a 100 bp ladder, lanes 2–7 PCR products amplified from the following tissues: 2 ovary, 3 cardiac muscle, 4 brain, 5 liver, 6 spleen, 7 skeletal muscle, and cell lines, 11 HeLa, 12 Vero, 13 CHO, 14 IBRS2, 15 RK, 16 BSC-1, 17 BK, 18 LT. Lanes 8 and 9 show control reactions containing either no primers (8) or an RNase treated sample (9).

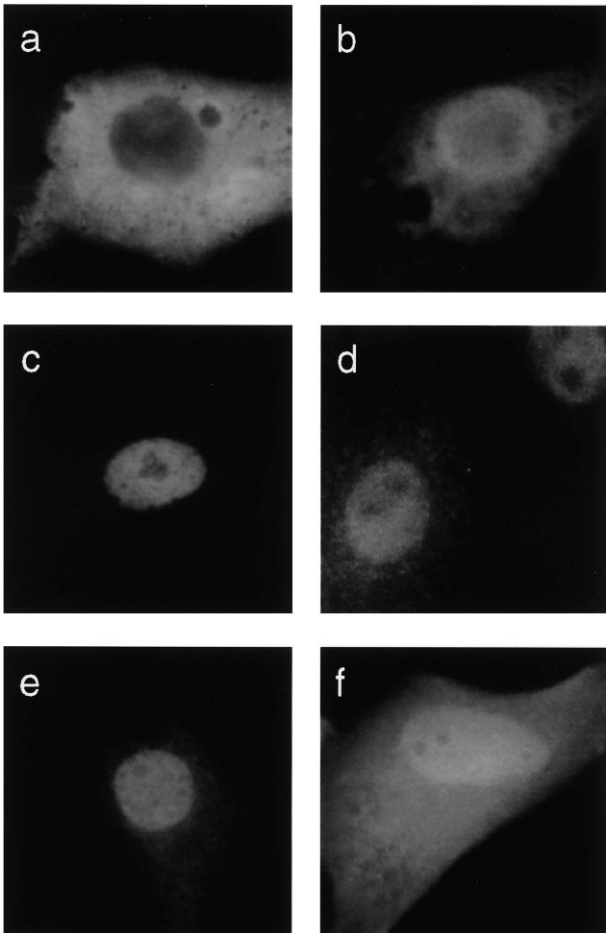

Fig. 4. Localisation of wild type and mutant HA-UBCv1 and SMCP proteins. Vero cells were infected with ASFV BA71V isolate and transfected with vp32-OP-HA-UBCv1 (panels a, b and c), vp32-OP-HA-UBCv1 (NLS Mut) (panel e), vp32-OP-HA-UBCv1 Δ C (panel f) or untransfected (panel d). At 16 h post infection the cells were fixed in 3% paraformaldehyde, permeabilised using 0.2% Triton X-100, probed with either a rat anti-HA monoclonal (1/100 dilution) (panels a, b, c, e and f) or rabbit anti-SMCp (panel d) sera (1/100 dilution) and with the appropriate FITC stained secondary antibody (rabbit anti-rat or goat anti-rabbit 1/100). Slides were visualised using a fluorescence microscope and photographed at 400-fold magnification.

(p32-OP-HA-UBCv1 NLS Mut) was transfected into ASFV infected Vero cells and the HA-UBCv1 detected by immunofluorescence using anti-HA antibodies. This mutant HA-UBCv1 still localised almost entirely in the nucleus of most transfected cells (Fig. 4e) showing that the VKKTVKKSLD sequence is not required for accumulation of UBCv1 in the nucleus.

UBC enzymes with acidic carboxy-terminal extensions similar to that of UBCv1 conjugate ubiquitin to their substrates in an E3 independent manner in vitro, indicating that they interact directly with their substrates [17]. The acidic C-termini of such enzymes are not only involved in substrate recognition but are also thought to dictate the intracellular localisation of the enzyme [17]. To test whether the UBCv1 acidic tail mediates the nuclear accumulation of UBCv1, sequences encoding residues 160–223 were deleted from the UBCv1 gene (p32-OP-HA-UBCv1ΔC). The plasmid expressing this mutant UBCv1 was transfected into ASFV infected Vero cells and the HA-UBCv1 detected by immunofluorescence. No cells were found with UBCv1 localised entirely in the nucleus, instead, in most cells equal amounts of UBCv1 were seen in the nucleus and the cytoplasm (Fig. 4f). This suggests that the acidic tail is required for an accumulation of UBCv1 in the nucleus.

4. Discussion

Many cellular proteins that control regulatory pathways are selectively degraded by the ubiquitin/proteasome pathway (reviewed in [6,7]). UBC enzymes are central to this process. One way to decipher the role of the UBCv1 enzyme encoded by ASFV is to determine protein(s) which UBCv1 targets for ubiquitination and thus degradation. The substrate(s) may be either host or virus encoded. We used the yeast two hybrid system to identify host proteins that bind to UBCv1 and which might, therefore, be enzyme substrates. Of the four clones encoding proteins which interacted specifically with UBCv1, three encoded ubiquitin. All UBC enzymes must interact with ubiquitin as they transfer the ubiquitin to substrate proteins; the demonstration that UBCv1 bound to ubiquitin provided evidence that the two hybrid screen had worked efficiently. One clone encoded a UBCv1 interacting protein that was homologous at the amino acid level to a fragment of the SMCX/Y proteins. This gene, designated SMCp, is very similar to the N-terminal region of the mammalian *SMCX/Y* genes (93% nucleotide identity) and RBBP2 (69% amino acid identity). The conservation of SMCp in different species, expression in many different tissues and sequence similarity with SMCX/Y genes, suggest it is the porcine homologue of these genes. Further evidence that UBCv1 binds to SMCp was provided by in vitro binding studies using the N-terminal 215 amino acids of SMCp expressed as a fusion with GST. This region of the SMCp protein contains the intact ARID DNA binding domain (residues 76–184), which is likely to be exposed on the surface of the intact protein and to be correctly folded since DNA binding domains can retain their function when expressed either alone or as fusions with other proteins. We therefore expect that UBCv1 would also bind specifically to this region of the intact SMCp protein. Due to the very large predicted size of SMCp (>1500 amino acids) it was technically not feasible to express the intact SMCp protein either in vitro or in *E. coli* and to test for direct binding of UBCv1 to the intact SMCp protein. Further evidence for interaction between UBCv1 and SMCp in infected cells was provided by immunofluorescence showing that the two proteins co-localise in the nucleus. UBCv1 is present almost entirely in the nucleus in most cells but is present in the cytoplasm in some cells. This suggests that UBCv1 shuttles between nucleus and cytoplasm. The signals which regulate this have not been defined. Mutagenesis of the UBCv1 sequence showed that the acidic C-terminal extension of UBCv1 is required for preferential accumulation of UBCv1 in the nucleus suggesting that interactions with this domain direct nuclear accumulation of UBCv1.

SMCX, SMCY, RBBP2 and Jumonji belong to the ARID family of proteins which have a conserved DNA binding domain involved in transcriptional regulation [13]. Within the ARID family tree, RBBP2 and SMCX cluster together suggesting that they are a subfamily and may have similar roles. In mice and humans, *SMCX* is expressed in all male and female tissues tested and escapes X-inactivation [1]. Con-

versely, *SMCY* is a male specific gene encoded by the Y chromosome and is also expressed in all male tissues tested. Kinetic studies of *SMCY* and *SMCX* expression in mice and primates showed that both genes are expressed early in life (at the two cell stage in mouse preimplantation embryos and at the four cell stage in primates [19]). Since SMCY and SMCX are expressed early in embryos and *SMCX* escapes X-inactivation, *SMC* genes may be essential and both copies necessary for normal function. The ARID domain is present within the region of SMCp which interacts with UBCv1 suggesting that this may be the interacting domain. The close similarity between this domain in SMCX/SMCY proteins and RBBP2 protein suggests that UBCv1 might also bind to the RBBP2 protein.

Interaction of SMCp with UBCv1 suggests that it may be a substrate for the enzyme and consequently be targeted for ubiquitin dependent degradation. Thus, the role of the UBCv1 enzyme during virus infection may be to interfere with SMCp function by targeting it for destruction. Since virus genes are usually captured from the host and can mimic the function of host genes, the implication is that there may be cellular proteins which have similar roles to that of UBCv1. By understanding how UBCv1 functions we may learn more about the function and regulation of ARID family proteins.

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