**In vitro and in vivo specificity of ubiquitination and degradation of STAT1 and STAT2 by the V proteins of the paramyxoviruses simian virus 5 and human parainfluenza virus type 2**

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Previous work has documented that the V protein of simian virus 5 (SV5) targets STAT1 for proteasome-mediated degradation, whilst the V protein of human parainfluenza virus type 2 (hPIV2) targets STAT2. Here, it was shown that the processes of ubiquitination and degradation could be reconstructed in vitro by using programmed rabbit reticulocyte lysates. Using this system, the addition of bacterially expressed and purified SV5 V protein to programmed lysates was demonstrated to result in the polyubiquitination and degradation of in vitro-translated STAT1, but only if human STAT2 was also present. Surprisingly, in the same assay, purified hPIV2 V protein induced the polyubiquitination of both STAT1 and STAT2. In the light of these in vitro results, the specificity of degradation of STAT1 and STAT2 by SV5 and hPIV2 in tissue-culture cells was re-examined. As previously reported, STAT1 could not be detected in human cells that expressed SV5 V protein constitutively, whilst STAT2 could not be detected in human cells that expressed hPIV2 V protein, although the levels of STAT1 may also have been reduced in some human cells infected with hPIV2. In contrast, STAT1 could not be detected, whereas STAT2 remained present, in a variety of animal cells, including canine (MDCK) cells, that expressed hPIV2 V protein, although the levels of STAT1 may also have been reduced in some human cells infected with hPIV2. In contrast, STAT1 could not be detected, whereas STAT2 remained present, in a variety of animal cells, including canine (MDCK) cells, that expressed the V protein of either SV5 or hPIV2. Thus, the V protein of SV5 appears to be highly specific for STAT1 degradation, but the V protein of hPIV2 is more promiscuous.

**INTRODUCTION**

In the absence of viral countermeasures, interferons (IFNs), which are produced either by cells in response to virus infection (IFN-α/β) or by subsets of activated lymphocytes (IFN-γ), both induce an antiviral state within cells and modulate the development of the immune response. Their antiviral effects are dependent upon the induction of a large number of cellular genes, some of which (including protein kinase R, 2′,5′-oligoadenylate synthetase and Mx proteins) have antiviral activity, whereas others sensitize cells for apoptosis or killing by cytotoxic T cells. IFNs induce transcription of IFN-responsive cellular genes by activating JAK/STAT pathways; IFN-α/β induce the formation of the ISGF3 transcription complex, which comprises STAT1, STAT2 and IRF-9 (p48), whilst IFN-γ induces the formation of the GAF transcription complex of STAT1 homodimers (Goodbourn et al., 2000).

Simian virus type 5 (SV5) and human parainfluenza virus type 2 (hPIV2) are classified within the genus Rubulavirus of the subfamily Paramyxovirinae of the family Paramyxoviridae (Lamb & Kolakofsky, 2001). It is now well-established that SV5 and hPIV2, and many other paramyxoviruses, at least partially circumvent the IFN response by blocking IFN signalling and IFN production (reviewed by Garcia-Sastre, 2004; Horvath, 2004; Nagai & Kato, 2004). In human cells, SV5 and mumps virus block IFN signalling by targeting STAT1 for proteasome-mediated degradation, whilst human hPIV2 targets STAT2 for degradation (Andrejeva et al., 2002b; Didcock et al., 1999a; Nishio et al., 2001; Parisien et al., 2001; Yokosawa et al., 2002; Young et al., 2000). As a consequence, SV5 inhibits both IFN-α/β and IFN-γ signalling, whilst in human cells, hPIV2 only blocks IFN-α/β signalling. Intriguingly, it has recently been reported that mumps virus, but not SV5, can also target STAT3 for degradation, although the biological significance of this has yet to be established (Ulane et al., 2003). The importance of IFN in controlling SV5 infections can be judged from studies in mice: SV5 fails to degrade STAT1 in murine cells and is non-pathogenic in normal and severe combined immunodeficient mice (which fail to make an adaptive immune response; Didcock et al., 1999b; Randall & Young, 1991), but is lethal in mice that lack STAT1 (He et al., 2002). These results indicate
that the relative lack of pathogenicity in normal mice can be at least partially attributed to the fact that SV5 fails to block IFN signalling in murine cells (Young et al., 2001).

Much has been learned about the molecular mechanisms by which SV5, mumps virus and hPIV2 target STAT1/2 for degradation. Of the virus proteins, the V protein is necessary and sufficient to mediate degradation (Andrejeva et al., 2002b; Didcock et al., 1999a; Parisien et al., 2002b). Degradation is not dependent upon IFN signalling and SV5 can induce degradation of both phosphorylated and non-phosphorylated STAT1 (Andrejeva et al., 2002b; Parisien et al., 2002b). Nevertheless, there appears to be an interdependence of STAT1 and STAT2 for degradation; thus, hPIV2 failed to degrade STAT2 in STAT1-deficient (U3A) cells and SV5 failed to degrade STAT1 in STAT2-deficient (U6A) cells (Parisien et al., 2002b). Furthermore, STAT2 was not degraded upon hPIV2 infection of human 2fTGH cells that constitutively expressed the V protein of SV5 (and thus lacked STAT1), whilst STAT1 was not degraded in 2fTGH cells expressing the hPIV2 V protein (which thus lacked STAT2) when infected by SV5 (Andrejeva et al., 2002b). Also, the V protein of SV5 can target murine STAT1 for degradation in murine cells if human STAT2 is co-expressed (i.e. the V protein of SV5 is unable to utilize murine STAT2 in the degradation complex; Parisien et al., 2002a).

The SV5 V protein interacts strongly with the 127 kDa subunit (DDB1) of the UV-damaged DNA-binding protein (DDB; Lin et al., 1998) and a clear role for DDB1 in the targeted degradation of STAT1 by the V protein of SV5 has now been firmly established (Andrejeva et al., 2002a; Leupin et al., 2003; Ulane & Horvath, 2002). In uninfected cells, DDB1 has been isolated as an interacting partner for several proteins, including Cullin 4a (Cul4a), which is associated with E3 ubiquitin ligase activity (Shiyanov et al., 1999). Indeed, it has recently been shown that DDB1 and Cul4a form part of an E3 ligase complex that regulates c-Jun activity (Wertz et al., 2004). Cullins function by serving as connectors between at least two groups of proteins. Firstly, they connect the ubiquitin E2 ligases to the E3 ligases and, secondly, they interact with substrate-recognition proteins to complete an E3 ligase complex that ubiquitinates the substrate protein specifically and hence targets it for proteasome-mediated degradation (for reviews on ubiquitination and proteasome-mediated degradation, see Glickman & Ciechanover, 2002; Jackson et al., 2000; Weissman, 2001). Suggestive evidence for a direct role of Cul4a in the targeted degradation of STAT1 by SV5 comes from the observations that Cul4a was co-immunoprecipitated with V and DDB1, and that treatment of cells with small interfering RNA to Cul4a affected the degradation of STAT1 slightly (Ulane & Horvath, 2002).

To begin to define further the importance of these interactions, we have been developing in vitro assays to address questions concerning the nature of the targeted ubiquitination of STAT1 or STAT2 by the V proteins of SV5 and hPIV2. Here, we report on the ubiquitination and degradation of STAT1 and/or STAT2 in rabbit reticulocyte lysates by the addition of bacterially expressed V proteins of SV5 and hPIV2. Using this system, we have also shown that whilst the ubiquitination of STAT1 by the V protein of SV5 appeared to be highly specific, the V protein of hPIV2 could ubiquitinate both STAT1 and STAT2 proteins. On the basis of these observations, we re-examined the ability of SV5 and hPIV2 to target STAT proteins for degradation in a wide variety of cells. These studies revealed that whilst SV5 always targeted STAT1 for degradation, hPIV2 was more promiscuous and the apparent specificity of degradation of STAT1 or STAT2 by hPIV2 was dependent upon the species from which the cells originated.

**METHODS**

**Cells, viruses and IFN.** MRC-5 [human fetal lung; European Collection of Cell Cultures (ECACC) #97112601], Hep2 (human larynx carcinoma; ECACC #96050601), HD-MY-Z [human Hodgkin’s lymphoma; Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) #ACC 346], KM-H2 (human Hodgkin’s lymphoma; DSMZ #ACC 8), HDF (human dermal fibroblasts; kindly provided by C. Hutchison, University of Durham, UK), Vero (African green monkey kidney; ECACC #84113001), NBL-6 (horse dermis skin fibroblast; ECACC #88032803), MDBK (bovine kidney, epithelial-like; ECACC #90050801), MDCK (canine kidney, epithelial-like; ECACC #85011435), RK13 (rabbit kidney, epithelial-like; ECACC #90021715) and murine BF (BALB/c mouse embryo fibroblasts) cells were cultured as monolayers in 25 or 75 cm2 tissue-culture flasks in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. All cell lines were negative for mycoplasma as screened by DAPI staining.

**Immunoblotting.** Immediately prior to harvesting, cells were washed twice with PBS and lysed into gel electrophoresis sample buffer. Cell lysates were then processed by sonication and heating at 100 °C for 5 min. Samples were subjected to SDS-PAGE, polypeptides were transferred to nitrocellulose membranes and STAT1 was detected by using a polyclonal anti-STAT1 antibody raised against the N-terminal 194 aa of the protein (cat. no. G16930; Transduction Laboratories). Protein–antibody interactions were visualized by enhanced chemiluminescence using horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amersham Biosciences).

**Plasmids.** Plasmids for T7 RNA polymerase-dependent in vitro translations were constructed by: (i) inserting a partial AflIII (5’ end)–BamHI fragment from pCEP4127 (a kind gift from Vesna Rapic-Otrin, University of Pittsburgh, USA) containing the entire ORF of DDB1 between the Ncol and BamHI sites of pGBK7 (Clontech) to make pGBK7.DDB1; (ii) inserting the entire ORF of human STAT2 flanked by Ncol (5’ end) and EcoRI sites, obtained by
RT-PCR, between the NcoI and EcoRI sites of pGBK7 to make pGBK7.hSTAT2. The plasmid pT7/STAT1z has been described previously (King & Goodbourn, 1998). The T7 polymerase-dependent Cul4a plasmid was a kind gift of Dr P. Zhou (Weill Medical College of Cornell University, NY, USA; Chen et al., 2001).

Cloning and purification of glutathione S-transferase (GST)–SV5 V fusion protein. The SV5 V gene was amplified from an existing clone by PCR using the following primers: forward, 5′-GGATCCCGAGATTCCgaaaaacctgattcagggccgaATGGAATCCACAG-3′, and reverse, 5′-GGCGGCCGCTCAAATTGCGACATGCGGATG-ATTG-3′, and ligated between the EcoRI and NolI restriction sites of pGEX-4T3 (Pharmacia). It should be noted that the forward primer contained coding sequences (lower-case letters) for the cleavage sequence of tobacco etch virus (TEV) protease. The plasmid was cloned into Escherichia coli B834 for optimum expression. GST–SV5 V protein was expressed by induction with 1 mM IPTG for 3 h at 30°C. After centrifugation, the bacterial cells were resuspended in buffer A (50 mM Tris/HCl (pH 7.5), 100 mM NaCl, 2 mM dithiothreitol (DTT)), lysed by sonication and the lysate was clarified by centrifugation at 30 000 r.p.m. for 30 min in a Beckman 42.1 rotor. Expressed GST–SV5 V protein was bound to a glutathione–agarose column (Sigma), eluted with glutathione by using standard protocols and used without further purification.

To purify SV5 V protein, GST–SV5 V was incubated overnight with recombinant TEV protease (Invitrogen), using a 50:1 ratio of protein : enzyme at room temperature with accompanying dialysis with recombinant TEV protease (Invitrogen), using a 50:1 ratio of protein : enzyme at room temperature with accompanying dialysis. After removal of GST by a second glutathione column, protein fractions were collected and analysed by SDS-PAGE. SV5 V protein-containing fractions were pooled and used in subsequent analyses.

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**STAT degradation and ubiquitination assays.** To demonstrate the degradation of STAT1 or STAT2, individual protein syntheses of STAT1, STAT2, DDB1 or Cul4a were made in reticulocyte lysates as described above. Depending on the experimental conditions, either L-[35S]methionine or unlabelled methionine was added to the individual transcription/translation mixtures. Five microlitres of the appropriate translation products were mixed together and to this was added 5 µl of a degradation buffer containing the following components: 240 mM Tris/HCl (pH 7.5), 30 mM MgCl2, 12 mM DTT, 3 mM ATP, 60 mM creatine phosphate, 20 U creatine phosphokinase ml−1 and 1-2 mg ubiquitin ml−1 (Sigma). Finally, 5 µl (10 µg) purified E. coli-expressed SV5 V protein in buffer A was also added where appropriate. The mixtures were made up to a total volume of 30 µl with additional reticulocyte lysate. To identify polyubiquitinated products, the protease inhibitor MG132 at a final concentration of 10 µM or the deubiquitination inhibitor ubiquitin aldehyde (UA) at a final concentration of 20 µg ml−1 was added to the mixtures. The mixtures were incubated at 37°C for 180 min and analysed by SDS-PAGE.

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![Fig. 1.](http://vir.sgmjournals.org)
STAT1. Additionally, GST–SV5 V was unable to precipitate Cullin 2 (Cul2) synthesized in the same system (Fig. 1a). However, it should be noted that, although GST–SV5 V was able to capture DDB1, STAT2 and Cul4a, these experiments did not address the question of whether the interactions with V were direct or indirect, as there were clearly many rabbit proteins present in the reticulocyte lysate background and these may have included rabbit DDB1, STAT proteins and Cul4a. Indeed, we were able to demonstrate by Western blot analysis that reticulocyte lysates contained rabbit DDB1 that could interact with GST–SV5 V (data not shown).

It has also been demonstrated previously that the V protein of an isolate of SV5 termed CPI is unable to effect the degradation of STAT1. Sequence analysis of the SV5(CPI) V protein revealed that there were only three amino acid substitutions in the N terminus of V compared with the wild-type virus. As SV5(CPI) V protein failed to interact with DDB1, it has been suggested that this may be the sole reason why SV5(CPI) fails to target STAT1 for degradation (Chatziandreou et al., 2002). It was therefore of interest to ascertain whether SV5(CPI) V protein interacts with Cul4a and STAT2. A GST–SV5(CPI) V fusion protein was constructed and tested in the in vitro system as described above. Fig. 1(b) shows that GST–SV5(CPI) V failed to precipitate DDB1, Cul4a or STAT2.

**Purified SV5 V protein expressed in E. coli causes the degradation of human STAT1 in vitro**

The results of the precipitation experiments described above established that SV5 V protein expressed in E. coli was capable of interacting with at least some of the cellular components associated with STAT1 degradation and it was thus of interest to determine whether STAT1 degradation could take place in vitro. In our initial experiments, STAT2, DDB1 and Cul4a were translated individually and mixed in combinations with in vitro-translated and 35S-methionine-labelled STAT1. It can be seen from Fig. 2(a) that STAT1 was degraded in the presence of SV5 V protein and human STAT2. However, in vitro-translated DDB1 and Cul4a could be omitted, possibly because these proteins are present within the reticulocyte lysates. To confirm that in vitro-translated human STAT2 was necessary, reticulocyte lysates containing L-[35S]methionine-labelled STAT1 were prepared for a degradation assay in the presence or absence of L-[35S]methionine-labelled STAT2 (Fig. 2b). These results showed that the presence of human STAT2 was required in this system for degradation to occur.

**Bacterially expressed SV5 V protein induces polyubiquitination of STAT1 in rabbit reticulocyte lysates**

It is well-established that degradation via the proteasome usually requires polyubiquitination of target proteins. Furthermore, it has been reported that expression of the V protein of SV5 can induce polyubiquitination of STAT1 in human cells (Ulane & Horvath, 2002; Ulane et al., 2003). To investigate whether polyubiquitination of STAT1 could be detected in vitro, a modification of the degradation assay (described in Methods) was used that included either the proteasome inhibitor MG132 or an inhibitor of deubiquitination (UA). These results demonstrated clearly that there was an accumulation of higher-molecular-mass L-[35S]methionine-labelled proteins in samples that contained either MG132 or UA (Fig. 3). Similarly, the presence of human STAT2 in the mixture was an absolute requirement for the accumulation of polyubiquitinated

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**Fig. 2.** (a) Human STAT1 is degraded in rabbit reticulocytes in the presence of SV5 V protein. Individual proteins were labelled metabolically, or not, with L-[35S]methionine (as shown) during synthesis by in vitro translation in reticulocyte lysates. Mixtures were prepared by using 5 μl of each lysate as indicated in the presence (+) or absence of SV5 V protein and made up to a total volume of 30 μl. Mixtures were then incubated for 180 min at 37°C and 10 μl was analysed by 10% SDS-PAGE. (b) Human STAT2 is required for SV5 V protein-mediated degradation of human STAT1 in rabbit reticulocytes. The experiment was carried out as described for (a), using mixtures of lysates as indicated (+).

**Fig. 3.** Requirements for in vitro ubiquitination of human STAT1 in rabbit reticulocyte lysates. Individual proteins were labelled metabolically, or not, with L-[35S]methionine (as shown) during synthesis by in vitro translation in reticulocyte lysates. Mixtures were prepared by using 5 μl of each lysate as indicated in the presence (+) or absence of SV5 V protein, MG132 or the deubiquitination inhibitor UA (as described in Methods) and made up to a total volume of 30 μl. Mixtures were incubated for 180 min at 37°C and 10 μl was analysed by SDS-PAGE using 4–12% gradient gels.
In the light of the latter results, we re-examined the degradation of STAT1 and STAT2 by SV5, hPIV2 and mumps virus in tissue-culture cells isolated from a variety of species (Fig. 5). In agreement with previously published results, STAT2 could not be detected in Hep2 or Vero cells infected with hPIV2, but STAT1 was clearly visible. However, in striking contrast, STAT1 was absent but STAT2 was detected (albeit in reduced amounts) in bovine MDBK cells and horse NBL-6 cells infected with hPIV2. Additional evidence that hPIV2 targeted STAT1 preferentially for degradation in some cells came from the observation that STAT1 was absent, but STAT2 was present, in MDCK cell lines that constitutively expressed the V protein of either SV5 or hPIV2 (Fig. 6). It was also noteworthy that, although hPIV2 targeted STAT2 preferentially for degradation in some human cell lines (Hep2 and HD-MY-Z), STAT1 levels were also severely reduced. By comparison, in all cell types examined, SV5 and mumps virus only targeted STAT1 for degradation.

**hPIV2 V polyubiquitinates both STAT1 and STAT2 in rabbit reticulocyte lysates**

Rather than target STAT1 for degradation, it has been reported that hPIV2 preferentially induces the degradation of STAT2 (Andrejeva et al., 2002b; Parisien et al., 2002b; Young et al., 2000). It was therefore of interest to ascertain whether this specificity of degradation was maintained in the rabbit reticulocyte ubiquitination and degradation assays. Purified SV5 or hPIV2 V protein was added to reticulocyte lysates containing L-[35S]methionine-labelled human STAT2 and unlabelled human STAT1, or L-[35S]methionine-labelled human STAT1 and unlabelled human STAT2. As expected, SV5 V protein induced the ubiquitination and degradation of STAT1 only. Surprisingly, however, in this assay, hPIV2 V protein induced the ubiquitination of both STAT1 and STAT2 (Fig. 4a). Furthermore, whilst STAT1 was clearly degraded by hPIV2 V protein, degradation of STAT2 was less obvious (Fig. 4b).

In the light of the latter results, we re-examined the degradation of STAT1 and STAT2 by SV5, hPIV2 and mumps virus by using the rabbit reticulocyte lysate system, which behaves in a functionally similar manner to previously reported in vivo systems (Andrejeva et al., 2002a; Didcock et al., 1999b; Parisien et al., 2002a; Young et al., 2000). We showed that SV5 V protein expressed in E. coli was functional for the induction of polyubiquitination and degradation of human STAT1 and that these processes were dependent on the presence of human STAT2. We further showed that in vitro degradation was inhibited by the proteasome inhibitor MG132 and that polyubiquitination was enhanced by the presence of the deubiquitination inhibitor UA using 5 μl of each lysate as indicated (+), together with, or without (control), the addition of purified SV5 or hPIV2 V protein (as shown). Mixtures were incubated for 180 min at 37 °C and 10 μl was analysed by SDS-PAGE using 4–12 % gradient gels. (b) Degradation of STAT1 and STAT2 by SV5 and hPIV2 V proteins. The experiment was carried out as described for (a), except that UA was left out of the mixtures.

**DISCUSSION**

In our investigations into the biochemical details of the degradation of STATs by rubulaviruses, we have further analysed the specific requirements for the degradation process. Here, we have reported the establishment of an in vitro assay using the rabbit reticulocyte lysate system, which behaves in a functionally similar manner to previously reported in vivo systems (Andrejeva et al., 2002a, b; Didcock et al., 1999b; Parisien et al., 2002a; Young et al., 2000). We showed that SV5 V protein expressed in E. coli was functional for the induction of polyubiquitination and degradation of human STAT1 and that these processes were dependent on the presence of human STAT2. We further showed that in vitro degradation was inhibited by the proteasome inhibitor MG132 and that polyubiquitination was enhanced by the presence of the deubiquitination inhibitor UA using 5 μl of each lysate as indicated (+), together with, or without (control), the addition of purified SV5 or hPIV2 V protein (as shown). Mixtures were incubated for 180 min at 37 °C and 10 μl was analysed by SDS-PAGE using 4–12 % gradient gels. (b) Degradation of STAT1 and STAT2 by SV5 and hPIV2 V proteins. The experiment was carried out as described for (a), except that UA was left out of the mixtures.

**Fig. 4.** (a) SV5 V protein induces the ubiquitination of STAT1 only, but hPIV2 V protein induces the ubiquitination of both STAT1 and STAT2 in rabbit reticulocyte lysates. Individual proteins were labelled metabolically, or not, with L-[35S]methionine (as shown) during synthesis by in vitro translation in reticulocyte lysates. Mixtures were prepared in the presence of the deubiquitination inhibitor UA using 5 μl of each lysate as indicated (+), together with, or without (control), the addition of purified SV5 or hPIV2 V protein (as shown). Mixtures were incubated for 180 min at 37 °C and 10 μl was analysed by SDS-PAGE using 4–12 % gradient gels. (b) Degradation of STAT1 and STAT2 by SV5 and hPIV2 V proteins. The experiment was carried out as described for (a), except that UA was left out of the mixtures.

Species of STAT1. Additionally, the accumulation of polyubiquitinated species of STAT1 in the presence of MG132 indicated that the degradation process was mediated by the proteasome. Similar experiments to those described above were also carried out by using a wheat-germ in vitro transcription/translation system instead of the reticulocyte lysate system. In these experiments, all of the known or suspected components of the degradation system (i.e. DDB1, Cul4a, STAT2 and L-[35S]methionine-labelled STAT1) were synthesized individually and mixed together with purified SV5 V protein and the degradation mix described above (see Methods). However, no degradation or polyubiquitination of STAT1 was observed in these reactions, even when the reaction mix was further supplemented with E1 and E2 ubiquitin ligases.
inhibitor UA. In addition, we demonstrated that hPIV2 V protein polyubiquitinated both STAT1 and STAT2 and, in this assay system, preferentially degraded STAT1.

The results presented here on the interaction of GST–SV5 V with in vitro–translated cellular proteins thought to be involved in targeted degradation of STAT1 generally support those of Parisien et al. (2002b), which showed that GST–SV5 V bound DDB1, Cul4a, STAT2 and STAT1 from human cell extracts. Whilst this current study and that of Parisien et al. (2002b) failed to determine whether or not these interactions were direct, in vitro–translated human STAT1 was not captured by GST–SV5 V. As STAT1 is the target molecule for degradation and was not polyubiquitinated or degraded in the absence of human STAT2, these results support the idea that STAT1 may be incorporated into the degradation complex by STAT2. The role of Cul4a in the system remains unclear. Clearly, Cul4a can interact with SV5 V protein, but it seems likely to do so via its association with DDB1. It has also been suggested that there may be some redundancy in the cullins that are involved in the STAT-degradation complex (Ulane & Horvath, 2002); however, the in vitro results presented here indicate that at least Cul2 is not able to interact either directly or indirectly with SV5 V protein and thus is unlikely to play any role in STAT degradation. Additionally, evidence is increasing for the specificity of interactions of cullins with substrate-recognition proteins in E3 ligases (Pintard et al., 2004), suggesting that it is perhaps unlikely that different cullins would be involved in the degradation of STATs. However, the presence of the rabbit proteins in the reticulocyte lysates, and our inability to substitute wheat-germ cell lysates in these assays, limit the amount of data that can be gained regarding cellular proteins involved in the degradation. To overcome this problem and to define the minimal requirements for ubiquitination of STAT proteins by rubulavirus V proteins, we are currently further developing in vitro assay systems based upon purified expressed proteins, rather than cell extracts.

Fig. 5. Analysis of in vivo degradation of STAT1 and STAT2 following SV5, hPIV2 or mumps virus infection of the indicated cell lines. Cells were infected with the appropriate viruses for 18–30 h and cell lysates were immunoblotted for STAT1 and STAT2. Over 95% of cells expressed viral antigens, as judged by immunofluorescence.

Given the observation that hPIV2 V degraded STAT1 preferentially in rabbit reticulocyte lysates, even in the presence of human STAT2, it was decided to re-examine the degradation of STAT1 and STAT2 by hPIV2 in a variety of tissue-culture cells. At the same time, SV5 and mumps virus were included to look for evidence of whether both tissue and species specificity might, in some cases, be determined by the ability of these viruses to degrade STAT proteins and hence block IFN signalling. As previously reported, hPIV2 degrades STAT2 preferentially in human cells (Andrejeva et al., 2002b). However, in some human cell types (e.g. Hep2 and HD-MY-Z), as well as inducing the degradation of STAT2, infection with hPIV2...
also led to a marked reduction in the amount of STAT1. Furthermore, in some animal cells (MDBK and NBL-6), infection with hPIV2 preferentially induced the degradation of STAT1. Additionally, in MDCK cells that expressed hPIV2 V protein constitutively, STAT1 was degraded fully but STAT2 was not. In contrast, SV5 and mumps virus were much more specific in that they always targeted STAT1 for degradation. The biological significance of these observations remains unclear. Nevertheless, the data suggest that the differences in specificity of STAT1 and STAT2 degradation shown by hPIV2 could be relatively minor and may reflect either the cellular background or even the relative concentrations of STAT1 and STAT2 within a cell (given that hPIV2 can induce the ubiquitination of both STAT1 and STAT2; once one STAT has been degraded, there will be no significant degradation of the other STAT, as both STAT1 and STAT2 are required in the degradation process). In this respect, it is relevant to note that complementation of STAT2-deficient human U6A cells with exogenous STAT2 led not only to the degradation of STAT2 by hPIV2, but also to a partial loss of endogenous STAT1, suggesting that the level of STAT2 within a cell may influence the apparent specificity of STAT degradation by hPIV2 (Parisien et al., 2002b). Also, whilst mumps virus degraded STAT1 in rabbit cells, SV5 did not. The inability of SV5 to degrade STAT1 in rabbit cells can be explained on the basis of the in vitro assays, in that the V protein of SV5 failed to ubiquitinate or degrade STAT1 in rabbit reticulocyte lysates unless human STAT2 was also added to the extracts; this is a situation similar to that observed in murine cells, where human STAT2 is required for SV5 to target STAT1 for degradation (Parisien et al., 2002a). Thus, although species specificity may be influenced by the ability of these viruses to target STAT1 or STAT2 for degradation, from these limited studies, there is no evidence that tissue specificity might be dictated in the same way, i.e. SV5, mumps virus and hPIV2 degraded either STAT1 or STAT2 in all the human cells tested, regardless of tissue of origin.

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REFERENCES


