Viruses must overcome diverse intracellular defense mechanisms to establish infection. The Vif (virion infectivity factor) protein of human immunodeficiency virus 1 (HIV-1) acts by overcoming the antiviral activity of APOBEC3G (CEM15), a cytidine deaminase that induces G to A hypermutation in newly synthesized viral DNA. In the absence of Vif, APOBEC3G incorporation into virions renders HIV-1 non-infectious. We report here that Vif counteracts the antiviral activity of APOBEC3G by targeting it for destruction by the ubiquitin-proteasome pathway. Vif forms a complex with APOBEC3G and enhances APOBEC3G ubiquitination, resulting in reduced steady-state APOBEC3G levels and a decrease in protein half-life. Furthermore, Vif-dependent degradation of APOBEC3G is blocked by proteasome inhibitors or ubiquitin mutant K48R. A mutation of highly conserved cysteines or the deletion of a conserved SLQ(Y/F)LA motif in Vif results in mutants that fail to induce APOBEC3G degradation and produce non-infectious HIV-1; however, mutations of conserved phosphorylation sites in Vif that impair viral replication do not affect APOBEC3G degradation, suggesting that Vif is important for other functions in addition to inducing proteasomal degradation of APOBEC3G. Vif is mono-ubiquitinated in the absence of APOBEC3G but is poly-ubiquitinated and rapidly degraded when APOBEC3G is coexpressed, suggesting that coexpression accelerates the degradation of both proteins. These results suggest that Vif functions by targeting APOBEC3G for degradation via the ubiquitin-proteasome pathway and implicate the proteasome as a site of dynamic interplay between microbial and cellular defenses.

Human immunodeficiency virus 1 (HIV-1) and other lentiviruses encode the vif (virion infectivity factor) gene, which is essential for the production of infectious virus (1, 2). Recent studies demonstrate that Vif counteracts the innate antiviral activity of APOBEC3G, also known as CEM15 (3–8). APOBEC3G, a member of the APOBEC family of cytidine deaminase-editing enzymes, is a potent DNA mutator that can be packaged into budding retroviruses (3, 6, 8, 9). After the initiation of reverse transcription, APOBEC3G edits minus-stranded viral cDNA, converting cytosine to uracil and affecting the stability and fidelity of newly synthesized cDNA (4–8). These mutations may initiate a DNA base excision repair pathway that compromises the structural integrity of the single-stranded viral DNA, resulting in the catastrophic failure of reverse transcription characteristic of Vif-defective (Δvif) viruses (10). Furthermore, the massive C to U conversion in the minus strand leads to G to A hypermutation in the viral genome, which affects subsequent stages of the viral life cycle and decreases viral fitness (6, 8). Thus, APOBEC3G editing likely contributes to sequence variation within viral populations, particularly the G to A mutational bias characteristic of HIV-1 (11, 12).

Vif is required in virus-producing cells during the late stages of infection (10, 13). Replication in “non-permissive” cells, such as primary T lymphocytes, macrophages, and certain T cell lines, is strictly dependent on Vif, whereas Vif is dispensable for replication in “permissive” cell lines, such as 293T cells (13, 14). APOBEC3G expression is restricted to non-permissive cells (3), whereas its expression in permissive cells confers a non-permissive phenotype (3, 6–9). In non-permissive cells, Δvif viruses can produce virions, but they fail to complete reverse transcription and cannot establish infection (10, 15, 16). The expression of Vif in trans in virus-producing cells but not target cells rescues this defect (17). Vif forms a complex with APOBEC3G, preventing its viral encapsidation, and thereby protects the viral genome from editing mutations (8). The interaction between Vif and APOBEC3G is species-specific (8), which may play a role in restricting the replication of HIV-1 to humans.

The mechanism by which Vif inhibits APOBEC3G encapsidation into HIV-1 is unclear. In this study we demonstrate that Vif counteracts the antiviral activity of APOBEC3G by promoting the ubiquitination and proteasomal degradation of APOBEC3G. By destabilizing APOBEC3G, Vif enhances viral infectivity, suggesting that the primary function of Vif is to target APOBEC3G for destruction. APOBEC3G also promotes polyubiquitination and degradation of Vif, suggesting a reciprocal relationship wherein coexpression accelerates the degradation of both proteins. These results suggest that Vif has evolved to target APOBEC3G for degradation and implicate the ubiquitin-proteasome pathway in the dynamic interplay of microbial and host defenses.
**Antibodies and Plasmids**—Antibodies used were rabbit anti-Vif (18), rat 1F7 anti-HA (Roche Applied Science), mouse M2 anti-FLAG (Sigma), mouse 9E10 anti-myc (Roche Applied Science), mouse anti-tubulin (Sigma), and rabbit anti-APOBEC3G (a gift of Greene and co-workers (19)). The HIV-1 NL4-3 proviral plasmid pNLX (pNL4-3/Xmal) has been described previously (20). pNLX/Vif was created by exchanging the Apal-EcoRI fragment from NL4-3/Vif (2) into pNL-XHIV (33) containing the Vif mutants T96A, S144A, and T188A have been previously described (21). pAPOBEC3G:HA was a gift of M. Malin (3). Epitope-tagged Vif was expressed from pNL1.A1.Vif-FLAG (gift of K. Strebel). Wild-type (WT) and mutant Vif expression plasmids were created in pCDNA1.(18). Ub, Ub$c_{\text{cat}}$, and HA-Ub were a gift of H. Gottlinger. mycUb and mycUb K48R were encoded by pRbG4-His6-mycUb (22).

**RESULTS**

**Vif Reduces Steady-state Levels of APOBEC3G**—As an initial approach to investigate how Vif counteracts the antiviral activity of APOBEC3G, we examined the effect of Vif on APOBEC3G expression. HA-tagged APOBEC3G was expressed in 293T cells, which do not express APOBEC3G endogenously (3). The 46-kDa HA-tagged APOBEC3G protein, in addition to minor 22- and 24-kDa species that may represent proteolytic degradation fragments of APOBEC3G, were detected by Western blotting (Fig. 1A). In the presence of Vif, the steady-state levels of APOBEC3G were markedly decreased (Fig. 1A). Similarly, endogenous APOBEC3G was depleted in a Hut78 cell line that stably expresses Vif (Fig. 1B). Co-transfection of increasing amounts of pNL1.A1.Vif-FLAG together with a constant amount of pCDNA1.Vif showed that Vif expression reduced APOBEC3G levels in a dose-dependent manner (Fig. 1C). This effect was specific for APOBEC3G because levels of the control protein tubulin were unchanged (Fig. 1C). A critical Vif:APOBEC3G ratio was required for the reduction of APOBEC3G levels (Fig. 1C, lanes 5 and 6). At lower Vif: APOBEC3G ratios, Vif had no detectable effect on APOBEC3G levels (Fig. 1C, lanes 2–4). Most subsequent experiments were therefore performed under conditions in which APOBEC3G levels were expected to be limiting. We then used loss-of-function Vif mutants to further explore the effect of Vif on APOBEC3G levels. The highly conserved cysteine residues at positions 114 and 133 and SLQ/Y/P/LA motif at residues 144–149 are required for Vif function and HIV-1 replication (18, 25).
Thr\textsuperscript{26}, Ser\textsuperscript{144}, and Thr\textsuperscript{188} are highly conserved phosphorylation sites in Vif. Mutation of Thr\textsuperscript{26} or Ser\textsuperscript{144} impairs but does not abolish viral replication (21, 26). In contrast to wild-type Vif, Vif\textsubscript{C114S/C133S} and the Vif3142–154 mutant that removes the SLQ(Y/F)LA motif showed a markedly reduced ability to decrease APOBEC3G levels (Fig. 1D), suggesting a relationship between the ability of Vif to reduce APOBEC3G levels and to support virus replication. However, the T96A, S144A, and T188A mutants retained the ability to decrease APOBEC3G levels (Fig. 1D). Thus, some mutations that reduce Vif function during viral replication still have the wild-type ability to decrease APOBEC3G levels. We then investigated whether Vif affects APOBEC3G mRNA levels in H9 cells infected with wild-type and \textit{vif}-deficient HIV-1 by reverse transcriptase PCR (Fig. 1E). APOBEC3G mRNA levels were unaffected by the presence of Vif. Furthermore, infection of H9 cells with either virus did not appear to increase APOBEC3G mRNA levels compared with uninfected cells. Thus, Vif did not alter APOBEC3G mRNA levels, suggesting that Vif decreases APOBEC3G protein levels at a post-translational step.

\textit{Vif Accelerates Degradation of APOBEC3G by the Ubiquitin-Proteasome Pathway}—The preceding experiments suggested that Vif might reduce APOBEC3G levels by promoting its degradation. Intracellular protein stability is regulated by lysosomal or proteasome-mediated degradation (27). The ubiquitin-proteasome pathway involves the ubiquitination of target proteins and subsequent degradation by the 26 S proteasome. The presence of high molecular mass species of APOBEC3G at \textasciitilde54 and 60 kDa (Fig. 1A) raised the possibility that it is covalently modified by ubiquitin. The proteasome inhibitors MG132 and clasto-lactacystine \textit{β}-lactone in combination markedly reduced the ability of Vif to induce the degradation of APOBEC3G (Fig. 2A). Similar results were observed over a range of proteasome inhibitor concentrations and combinations.\textsuperscript{2} In contrast, the lysosome inhibitor chloroquine had no effect on APOBEC3G stability (Fig. 2C). The expression of APOBEC3G in the presence of myc-tagged ubiquitin resulted in a shift of the higher molecular weight species of APOBEC3G, suggesting that APOBEC3G is ubiquitinated.\textsuperscript{2} This was confirmed by Western blot analysis of immunoprecipitated APOBEC3G with anti-myc antibody, which showed a high molecular weight species typical of polyubiquitination (Fig. 2B, top). Coexpression of Vif dramatically increased polyubiquitination of APOBEC3G (Fig. 2B, top). The effect of Vif on APOBEC3G ubiquitination was most readily detected at high APOBEC3G expression levels (Fig. 2B, middle). However, APOBEC3G ubiquitination was also observed at lower levels of APOBEC3G expression that are destabilized by Vif (Figs. 1A and 2A). These findings indicate that Vif promotes the polyubiquitination and proteasome-mediated degradation of APOBEC3G.

Polyubiquitination typically results in proteolysis of the substrate protein via the proteasome. Treatment of cells with MG132/\textit{β}-lactone increased the levels of polyubiquitinated APOBEC3G, suggesting that APOBEC3G is rapidly degraded by the proteasome (Fig. 2C, top). This was further supported by experiments using the ubiquitin mutant K48R. Multiquitin chains linked via Lys48 are the principal signals for proteasomal degradation. UbK48R is a polyubiquitin chain terminating that reduces the efficiency of proteasome-mediated degradation and stabilizes polyubiquitinated substrates (28). When coexpressed with APOBEC3G and Vif, UbK48R enhanced the accumulation of polyubiquitinated APOBEC3G (Fig. 2C, top). The accumulation of high molecular weight conjugates in the presence of UbK48R suggests the attachment of ubiquitin to multiple sites within APOBEC3G and/or the incorporation of endogenous wild-type ubiquitin into the mutant polyubiquitin chains. The accumulation of polyubiquitinated APOBEC3G is probably the consequence of disrupted proteasomal targeting signals or the dynamic remodeling of polyubiquitin chains that occur in the presence of the K48R mutant (28, 29). In contrast to the effects of proteasome inhibitors, chloroquine had no significant effect. The proteasome inhibitors and ubiquitin mutants did not affect steady-state levels of APOBEC3G in the absence of Vif (Fig. 2C, bottom), suggesting that Vif accelerates the degradation of APOBEC3G by the ubiquitin-proteasome pathway.

Given that Vif decreases APOBEC3G levels and increases its ubiquitination, we investigated the effect of Vif on the half-life of APOBEC3G in virus-producing cells. APOBEC3G was expressed with WT or \textit{Δvif} proviruses and analyzed by pulse-chase metabolic labeling. APOBEC3G displayed a biphasic decay characterized by rapid degradation in the early phase, followed by a slow decay of a relatively stable APOBEC3G population during the late phase (Fig. 2D). The half-life of APOBEC3G during the early phase was significantly decreased from approximately 29 min in the absence of Vif to 18 min in its presence. Proteasome inhibitors restored the half-life of APOBEC3G in the presence of WT virus to that of APOBEC3G with \textit{Δvif} virus.\textsuperscript{2} The stable population of APOBEC3G detected during the late phase appeared to be unaffected by Vif, which may account for the inability of Vif to induce complete degradation of APOBEC3G (Fig. 1A). These results demonstrate two populations of APOBEC3G that differ in their susceptibility to down-modulation by Vif and are degraded at different rates and suggest that Vif decreases the half-life of the susceptible population during early phase decay by promoting its degradation via the ubiquitin-proteasome pathway.

\textit{Vif and APOBEC3G Form a Complex}—We next investigated whether Vif interacts with APOBEC3G or promotes its degradation through an indirect mechanism. To determine whether Vif and APOBEC3G interact in a complex, we performed reciprocal co-immunoprecipitation assays after expression of Vif and/or HA-tagged APOBEC3G in 293T cells. A significant amount of APOBEC3G could be specifically co-immunoprecipitated with wild-type Vif antibody (Fig. 3A, top). In a reciprocal experiment, Vif was co-immunoprecipitated by an anti-HA antibody (Fig. 3A, middle). No significant immunoprecipitation of either protein was observed when expressed in the absence of each other. Consistent with the preceding experiments, levels of APOBEC3G were reduced when APOBEC3G was coexpressed with Vif (Fig. 3A, bottom). We also performed experiments with purified recombinant proteins to determine whether Vif and APOBEC3G can interact directly. APOBEC3G was precipitated with GST-Vif but not GST or the truncated GST-Vif (40–160) (Fig. 3B). These results suggest that Vif and APOBEC3G interact directly and form a complex \textit{in vivo}, which accelerates the degradation of APOBEC3G via the ubiquitin-proteasome pathway.

\textit{Proteasomal Degradation of Vif Is Enhanced by APOBEC3G}—To further investigate the relationship between Vif-dependent degradation of APOBEC3G and viral infectivity, we tested the infectivity of WT and \textit{Δvif} virus produced in the presence of increasing amounts of APOBEC3G. Increasing expression of APOBEC3G dramatically reduced the infectivity of \textit{Δvif} virus in a dose-dependent manner (Fig. 4A) as previously reported (3). However, a smaller yet significant decrease in the infectivity of WT HIV-1 was also observed (Fig. 4A). Comparing APOBEC3G expression in 293T cells producing WT or \textit{Δvif} virus demonstrated lower levels of APOBEC3G in the presence of the WT virus. Surprisingly, we found that steady-state levels

\textsuperscript{2} A. Mehle and D. Gabuzda, unpublished observations.
of Vif were affected by APOBEC3G expression. High expression of APOBEC3G caused a significant reduction in Vif levels in parallel with a reduction in the infectivity of WT virus (Fig. 4A). These data suggest a reciprocal relationship wherein Vif and APOBEC3G influence the steady-state levels of each other.

To investigate the mechanism by which APOBEC3G decreases the steady-state levels of Vif, we examined whether Vif is ubiquitinated. A higher molecular mass 32-kDa form of Vif was observed in transfected 293T cells (Figs. 1A and 4B). The 8-kDa increase in size suggested covalent modification by ubiquitin. Coexpression of Vif with epitope-tagged ubiquitin resulted in a shift in size of the higher molecular weight form (Fig. 4B, top left). Western blotting of immunoprecipitated Vif with an antibody that recognizes the HA epitope tag on ubiquitin confirmed that Vif is covalently modified by the attachment of ubiquitin (Fig. 4B, top right). In the absence of APOBEC3G, the predominant ubiquitinated species of Vif appeared to be modified by monoubiquitin (Fig. 4B, top, data not shown). The chain-terminating mutant UbK48R had no effect on the ubiquitination profile of Vif, consistent with a monoubiquitinated species (Fig. 4B, bottom). Furthermore, treatment of cells with proteasome inhibitors failed to substantially increase the levels of ubiquitinated Vif (Fig. 4B, bottom). These results demonstrate that in the absence of APOBEC3G a minor fraction of Vif is monoubiquitinated. In the presence of APOBEC3G, however, the amount of monoubiquitinated Vif was increased (Fig. 4C). Furthermore, we observed a dramatic increase in polyubiquitinated Vif when APOBEC3G was present. Coexpression of Vif and APOBEC3G with the mutant UbK48R showed increased amounts of ubiquitinated Vif compared with that observed with WT ubiquitin (Fig. 4C). We next examined the effects of APOBEC3G on the half-life of Vif. Pulse-chase metabolic labeling showed that Vif is a relatively stable protein with a half-life of ~90 min (Fig. 4D). However, in
Ubiquitin can then be directly conjugated to a protein by ubiquitin-protein ligases, which catalyze the transfer of ubiquitin to a target protein. This process is often referred to as ubiquitination. Ubiquitination is a tightly regulated cellular mechanism that plays a role in various biological processes, including the regulation of protein stability, signaling pathways, and the response to stress and infection.

**DISCUSSION**

APOBEC3G is a DNA-editing enzyme with antiviral activity that is overcome by Vif (5–7, 9). Vif blocks the encapsidation of APOBEC3G into HIV-1 virions (6), but the mechanism of action by which this occurs is unclear. Here we report that Vif counteracts APOBEC3G by targeting it to the ubiquitin pathway for proteasomal degradation. Vif forms a complex with APOBEC3G through a direct physical interaction and enhances APOBEC3G ubiquitination, resulting in destabilization of APOBEC3G. Vif accelerates the degradation of both ectopically expressed and endogenous APOBEC3G and recently has been shown to deplete APOBEC3G levels in HIV-1-infected cells (19). The Vif mutants C114S/C133S and Δ142–154 that cannot support infection fail to induce degradation of APOBEC3G. Mutation of the Thr96 and Ser144 phosphorylation sites within Vif impairs viral replication (26) but does not affect APOBEC3G degradation, suggesting that Vif may be important for other functions in addition to inducing degradation of APOBEC3G by the proteasome. These findings suggest that the primary mechanism of action by which Vif neutralizes APOBEC3G is by promoting its degradation via the ubiquitin-proteasome pathway, thereby blocking its incorporation into virions. However, our results also imply that an additional mode of action, such as APOBEC3G binding and sequestration, leading to the inhibition of APOBEC3G incorporation into virions (8) or the recruitment of APOBEC3G away from sites of virus assembly may contribute to the functional activity of Vif and the generation of infectious virus.

Ubiquitination is a tightly regulated cellular mechanism that can control the activity, localization, and turnover rates of many cellular proteins (27). The ubiquitin machinery consists of an enzyme cascade that activates ubiquitin and ultimately transfers it to target proteins. An E1 ubiquitin-activating enzyme transfers ubiquitin to an E2 ubiquitin-conjugating enzyme. Ubiquitin can then be directly conjugated to a protein substrate by the E2, but more commonly an E3 ubiquitin ligase is required to covalently attach ubiquitin to the e-amino group of a lysine residue on the substrate. The E3 ubiquitin ligase conveys the exquisite targeting specificity of the ubiquitination machinery. Vif forms a complex with and accelerates the ubiquitination and degradation of a population of APOBEC3G, raising the possibility that Vif may directly ubiquitinate APOBEC3G. However, Vif has no sequence homology to known E3 enzymes. Furthermore, Vif does not appear to have intrinsic ubiquitin ligase activity, because attempts to reconstitute APOBEC3G degradation in vitro using Vif and purified components of the ubiquitin machinery were unsuccessful. Therefore, it seems unlikely that Vif is the enzyme directly responsible for APOBEC3G ubiquitination. This was confirmed by the recent work of Yu et al. (30), which demonstrated that Vif function is dependent on the enzymatic activity of the ElonginB-ElonginC-Cullin5 ubiquitin ligase complex and suggested that Vif may function as an F-box-like protein by acting as the specificity factor that links APOBEC3G to the ElonginB-ElonginC-Cullin5 complex. We found that Vif is monoubiquitinated in the absence of APOBEC3G, although monoubiquitination of Vif does not appear to target the protein for degradation. Rather, monoubiquitination may affect its structure, location, or activity (31). We previously demonstrated that mutation of lysines within the Vif C terminus disrupts membrane targeting of Vif and virus infectivity (32). Whether these lysines serve as ubiquitin conjugation sites remains to be determined. On expression of APOBEC3G a fraction of Vif is polyubiquitinated and rapidly degraded; thus, coexpression of APOBEC3G and Vif decreases the stability of both proteins. This finding, together with the observation that Vif and APOBEC3G form a complex in vitro and in vivo (Fig. 3) and the demonstration that Vif mutants that fail to bind APOBEC3G do not decrease APOBEC3G levels (33), raises the possibility that these two proteins may be coordinately degraded as a complex. However, further studies are required to determine whether Vif and APOBEC3G are degraded within the same complex or independently.

Our findings suggest that the ratio between Vif and APOBEC3G is crucial for maximal effects of Vif on APOBEC3G degradation and viral infectivity. In infected cells, Vif is expressed at high levels, approaching those of Gag (34). In contrast, APOBEC3G appears to be expressed at very low levels in non-permissive cells (19). Thus, it appears that HIV-1 conquers the cellular defenses of APOBEC3G by expressing the high levels of Vif that are needed to achieve a Vif:APOBEC3G ratio that leads to APOBEC3G degradation. A difference in Vif:APOBEC3G ratios may explain why our results differ from those previously reported by Mariani et al. (8), who reported that Vif had no significant effect on the steady-state levels or half-life of APOBEC3G. While this manuscript was under review, other studies were published that support our findings of a Vif-dependent decrease in APOBEC3G half-life and steady-state levels (19, 30, 33, 35). Compared with these studies, our study reaches the novel conclusion that there is a reciprocal destabilizing relationship between Vif and APOBEC3G because coexpression of APOBEC3G promotes Vif ubiquitination and reduces its half-life. These results differ from those reported by Stopak et al. (19) and Marin et al. (33) who did not observe that APOBEC3G destabilizes Vif. These discrepancies may be the result of differences in the ratio of APOBEC3G:Vif, which strongly influences the ability to detect this phenomenon. Furthermore, we performed experiments to determine the effect of APOBEC3G on the half-life and ubiquitination of Vif, whereas these other studies did not. Whether the levels of APOBEC3G used in Fig. 4 are ever approached in vivo during certain conditions that increase APOBEC3G expression, such as mitogenic stimulation (19), remains to be determined.
theless, we observed that only a fraction of Vif is ubiquitinated and destabilized by coexpression of APOBEC3G, possibly because of limiting amounts of APOBEC3G expressed in these cells or the differential localization of a fraction of both proteins.

In the absence of Vif, we found that APOBEC3G has a relatively short half-life of only 29 min during its early phase of decay. The half-life of APOBEC3G is highly dependent on its level of expression; but in the absence of Vif, the half-life of APOBEC3G increases to around 50 min or 5–6 h when we transfected 0.5 or 5 μg, respectively, of pAPOBEC3G:HA plasmid instead of 0.1 μg.2 Endogenous APOBEC3G is expressed at very low levels (19), so its half-life is expected to be short under physiological conditions. Given the editing capabilities of APOBEC3G, its rapid turnover may represent a cellular mechanism to tightly regulate the expression of a potential DNA mutator. Inducing expression of APOBEC3G might bolster the antiviral response and decrease the production of infectious virus but may also be deleterious to the overall fitness of the cell. For example, unregulated expression of the related cytidine deaminase APOBEC1 is oncogenic in animal models (36).

APOBEC3G displayed a biphasic decay characterized by rapid degradation in the early phase followed by slow degradation during the late phase. The biphasic decay could be because of differential degradation of APOBEC3G populations localized to distinct compartments within the cell. The majority of APOBEC3G is cytoplasmic with a minor fraction localized to the nucleus (19). Vif, a cytoplasmic protein, was capable of
Vif-dependent Degradation of APOBEC3G

reduced the half-life of APOBEC3G only during early phase decay, consistent with our finding that high levels of Vif did not induce complete degradation of APOBEC3G. Nonetheless, virus with full infectivity was produced. Thus, there appears to be a population of APOBEC3G that is resistant to Vif-dependent degradation and does not influence viral infectivity. Whether this represents the nuclear fraction or a cytoplastic fraction localized away from sites of virus assembly remains to be determined.

We propose that Vif functions as an adaptor molecule, recruiting APOBEC3G to the ubiquitin-proteasome machinery. Other viral proteins are known to subvert the ubiquitination machinery by a similar mechanism. The papillomavirus E6 oncoprotein bridges the tumor suppressor p53 to the ubiquitin ligase E6-AP (37). The HIV-1 Vpu protein scaffolds a ternary complex with CD4 and the E3 ligase E6-AP (37). The HIV-1 Vpu protein scaffolds a ternary complex with CD4 and the E3 ligase E6-AP (37). The HIV-1 Vpu protein scaffolds a ternary complex with CD4 and the E3 ligase E6-AP (37).

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