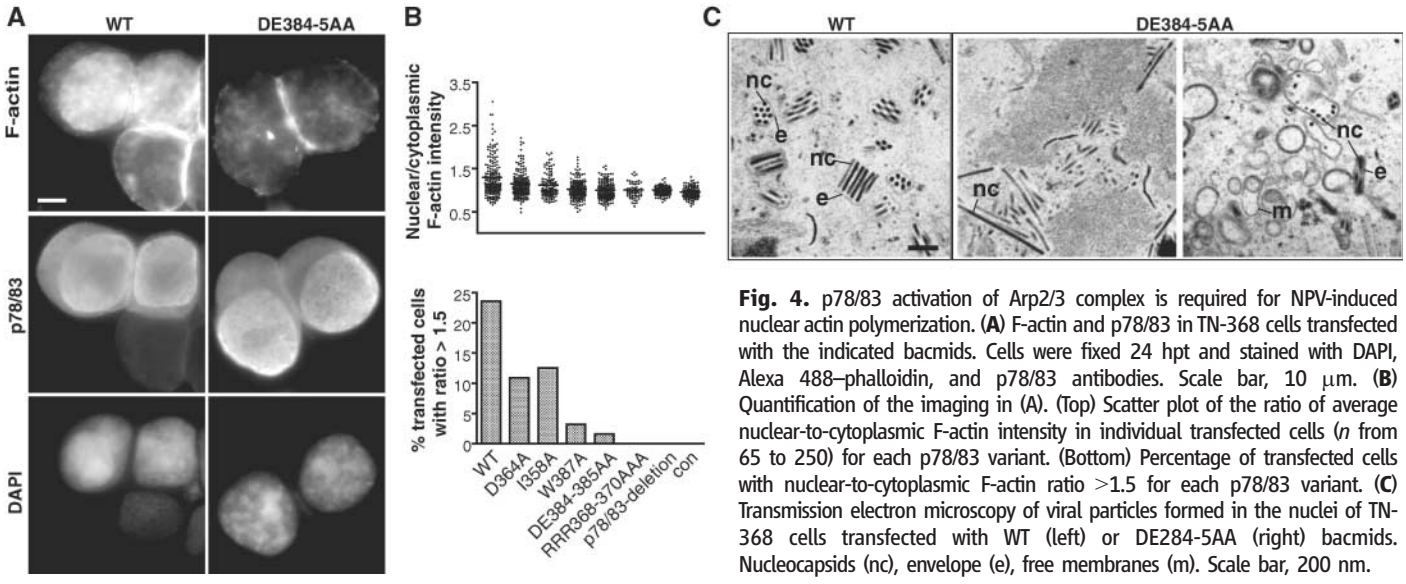

Fig. 4. p78/83 activation of Arp2/3 complex is required for NPV-induced nuclear actin polymerization. (**A**) F-actin and p78/83 in TN-368 cells transfected with the indicated bacmids. Cells were fixed 24 hpt and stained with DAPI, Alexa 488–phalloidin, and p78/83 antibodies. Scale bar, 10 μm. (**B**) Quantification of the imaging in (A). (Top) Scatter plot of the ratio of average nuclear-to-cytoplasmic F-actin intensity in individual transfected cells (*n* from 65 to 250) for each p78/83 variant. (Bottom) Percentage of transfected cells with nuclear-to-cytoplasmic F-actin ratio >1.5 for each p78/83 variant. (**C**) Transmission electron microscopy of viral particles formed in the nuclei of TN-368 cells transfected with WT (left) or DE284-5AA (right) bacmids. Nucleocapsids (nc), envelope (e), free membranes (m). Scale bar, 200 nm.

for nuclear actin polymerization in coordinating nucleocapsid morphogenesis and membrane-capsid interactions during virion assembly.

AcMNPV has evolved exquisite control over the actin cytoskeleton of its host cell, manipulating both activity and localization of the Arp2/3 complex to promote dynamic nuclear actin polymerization that is essential for proper virion processing and infectivity. Given the conservation of p78/83 among lepidopteran NPVs (*13*, *17*), it is likely that these viruses use the same mechanism for nuclear actin polymerization. It seems quite possible that other unrelated pathogens have also evolved similar strategies for exploiting nuclear actin (*18*). Because pathogens rarely invent cell biological processes, preferring to adapt existing pathways to their own needs, we suggest that AcMNPV may have co-opted existing nuclear functions and regulatory mechanisms of actin to facilitate its replication.

References and Notes

1. T. A. Kost, J. P. Condreay, D. L. Jarvis, *Nat. Biotechnol.* **23**, 567 (2005).
2. C. A. Charlton, L. E. Volkman, *J. Virol.* **65**, 1219 (1991).
3. C. A. Charlton, L. E. Volkman, *Virology* **197**, 245 (1993).
4. T. Ohkawa, A. R. Rowe, L. E. Volkman, *J. Virol.* **76**, 12281 (2002).
5. L. E. Volkman, P. A. Goldsmith, R. T. Hess, *Virology* **156**, 32 (1987).
6. L. E. Volkman, *Virology* **163**, 547 (1988).
7. R. T. Hess, P. A. Goldsmith, L. E. Volkman, *J. Invertebr. Pathol.* **53**, 169 (1989).
8. D. McDonald, G. Carrero, C. Andrin, G. de Vries, M. J. Hendzel, *J. Cell Biol.* **172**, 541 (2006).
9. M. T. Bohnsack, T. Stuven, C. Kuhn, V. C. Cordes, D. Gorlich, *Nat. Cell Biol.* **8**, 257 (2006).
10. T. Pederson, U. Aebi, *Mol. Biol. Cell* **16**, 5055 (2005).
11. J. A. Theriot, T. J. Mitchison, *Nature* **352**, 126 (1991).
12. M. D. Welch, R. D. Mullins, *Annu. Rev. Cell Dev. Biol.* **18**, 247 (2002).
13. L. M. Machesky, R. H. Insall, L. E. Volkman, *Trends Cell Biol.* **11**, 286 (2001).
14. R. D. Possee *et al.*, *Virology* **185**, 229 (1991).
15. S. C. Panchal, D. A. Kaiser, E. Torres, T. D. Pollard, M. K. Rosen, *Nat. Struct. Biol.* **10**, 591 (2003).
16. J. B. Marchand, D. A. Kaiser, T. D. Pollard, H. N. Higgs, *Nat. Cell Biol.* **3**, 76 (2001).
17. Y. Nie *et al.*, *Virus Res.* **116**, 129 (2006).
18. B. Feierbach, S. Piccinotti, M. Bisher, W. Denk, L. W. Enquist, *PLoS Pathog.* **2**, 0763 (2006).

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Supporting Online Material

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S6K1- and βTRCP-Mediated Degradation of PDCD4 Promotes Protein Translation and Cell Growth

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The tumor suppressor programmed cell death protein 4 (PDCD4) inhibits the translation initiation factor eIF4A, an RNA helicase that catalyzes the unwinding of secondary structure at the 5′ untranslated region (5′UTR) of messenger RNAs (mRNAs). In response to mitogens, PDCD4 was rapidly phosphorylated on Ser[67] by the protein kinase S6K1 and subsequently degraded via the ubiquitin ligase SCF$^{\beta TRCP}$. Expression in cultured cells of a stable PDCD4 mutant that is unable to bind βTRCP inhibited translation of an mRNA with a structured 5′UTR, resulted in smaller cell size, and slowed down cell cycle progression. We propose that regulated degradation of PDCD4 in response to mitogens allows efficient protein synthesis and consequently cell growth.

The proteolysis of many cellular regulators is controlled by SCF (SKP1–CUL1–F-box protein) ubiquitin ligases (*1*). In humans, there are 68 SCF ligases (*2*), each characterized by a different F-box protein subunit that provides specificity by directly recruiting the substrate. Mammals express two distinct paralogs of the F-box protein βTRCP (βTRCP1 and βTRCP2), with biochemical properties that are indistinguishable. We will therefore use the term βTRCP to refer to both, unless specified.

To identify new substrates of the SCF$^{\beta TRCP}$ ubiquitin ligase, we used an immunopurification strategy that enriches for ubiquitylated substrates, followed by mass spectrometry analysis (*3*). In addition to peptides derived from known

$SCF^{\beta TRCP}$ substrates, we also recovered three peptides corresponding to programmed cell death protein 4 (PDCD4), not previously identified as an $SCF^{\beta TRCP}$ substrate.

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PDCD4 is a tumor suppressor protein that is lost in certain aggressive human carcinomas and whose expression inhibits transformation in cultured cells and in a mouse model of tumorigenesis [reviewed in (*4*) and (*5*)]. PDCD4 binds the eukaryotic translation initiation factor (eIF) 4A (*6*–*8*), an RNA helicase that catalyzes the unwinding of mRNA secondary structure at the 5′ untranslated region (5′UTR). PDCD4 also binds eIF4G (*7*, *9*) and is thought to prevent translation by competing with eIF4G for binding to eIF4A, or inhibiting eIF4A's helicase activity, or both.

Because protein translation is stimulated by mitogens, we examined their impact on the abundance of PDCD4. Human T98G cells (revertants from T98 glioblastoma cells that acquired the property to accumulate in G_0/G_1 in low serum) and normal human fibroblasts were deprived of serum for 72 hours and then reactivated by addition of serum. In mitogen-deprived cells, the overall amount of PDCD4 increased, but after mitogen stimulation, it rapidly decreased (Fig. 1A and fig. S1). Treatment of the cells with the proteasome inhibitor MG132 prevented the disappearance of PDCD4 (Fig. 1B).

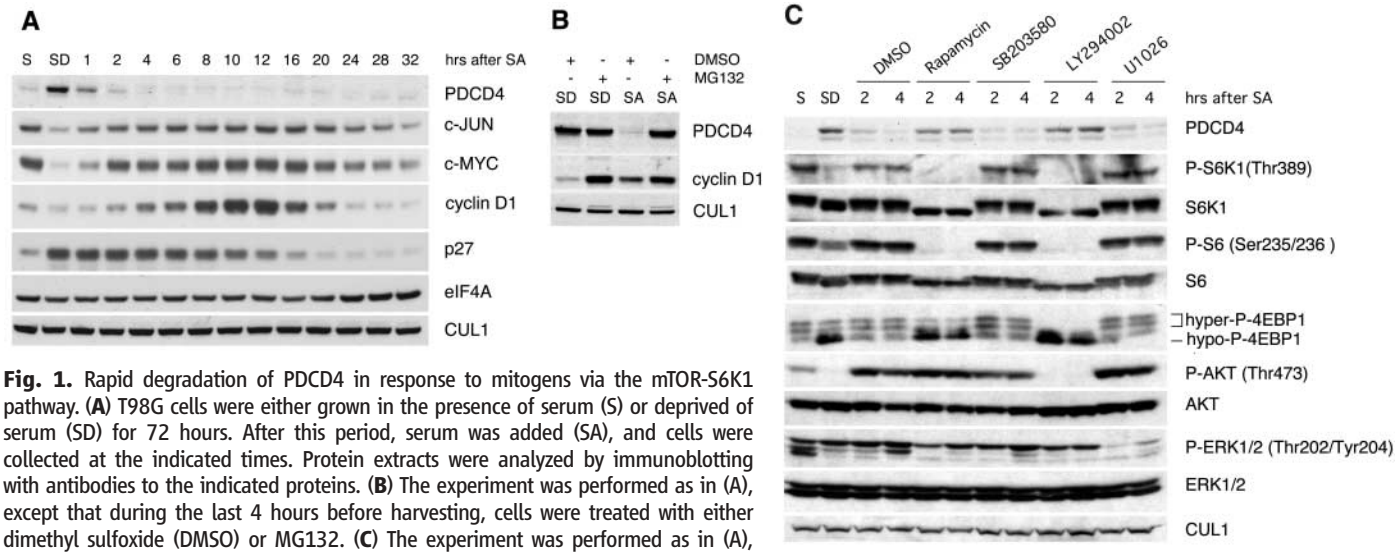


Fig. 1. Rapid degradation of PDCD4 in response to mitogens via the mTOR-S6K1 pathway. (**A**) T98G cells were either grown in the presence of serum (S) or deprived of serum (SD) for 72 hours. After this period, serum was added (SA), and cells were collected at the indicated times. Protein extracts were analyzed by immunoblotting with antibodies to the indicated proteins. (**B**) The experiment was performed as in (A), except that during the last 4 hours before harvesting, cells were treated with either dimethyl sulfoxide (DMSO) or MG132. (**C**) The experiment was performed as in (A), except that, together with serum, various drugs were added, as indicated.

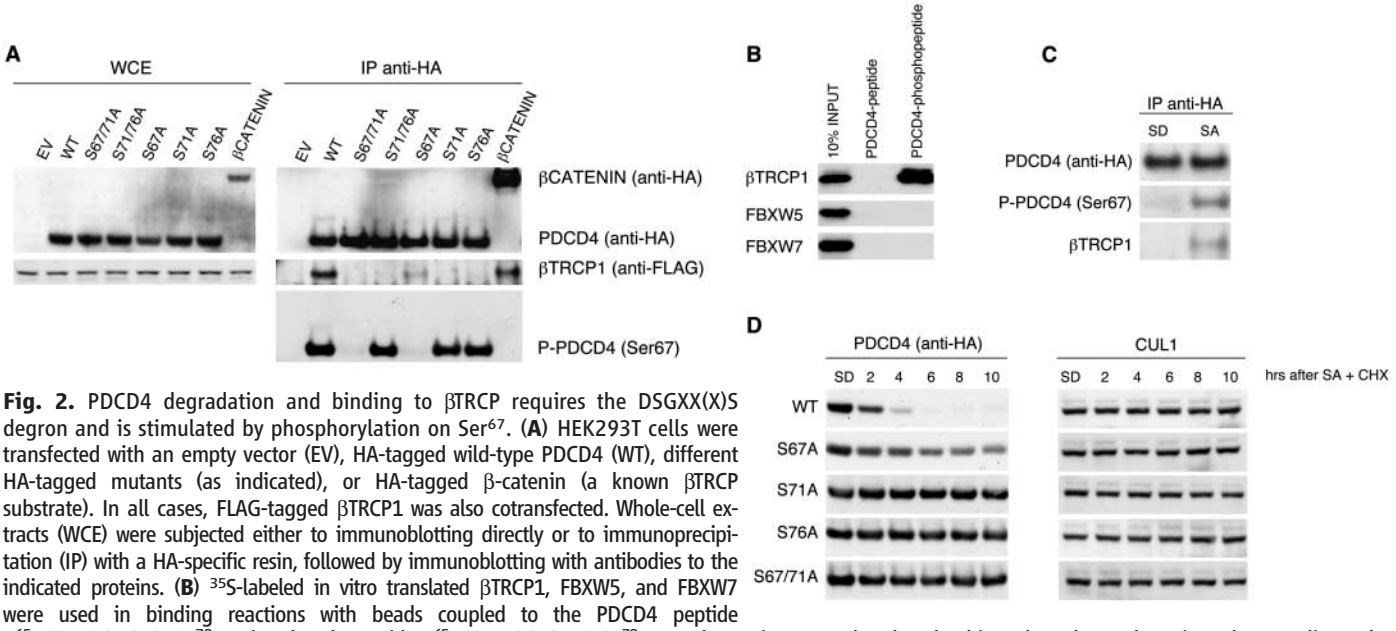


Fig. 2. PDCD4 degradation and binding to βTRCP requires the DSGXX(X)S degron and is stimulated by phosphorylation on Ser^{67}. (**A**) HEK293T cells were transfected with an empty vector (EV), HA-tagged wild-type PDCD4 (WT), different HA-tagged mutants (as indicated), or HA-tagged β-catenin (a known βTRCP substrate). In all cases, FLAG-tagged βTRCP1 was also cotransfected. Whole-cell extracts (WCE) were subjected either to immunoblotting directly or to immunoprecipitation (IP) with a HA-specific resin, followed by immunoblotting with antibodies to the indicated proteins. (**B**) ^{35}S-labeled in vitro translated βTRCP1, FBXW5, and FBXW7 were used in binding reactions with beads coupled to the PDCD4 peptide K^{65}NSSRDSGRGDSVSD^{79} or the phosphopeptide K^{65}NSSRDpSGRGDpSVSD^{79}. Bound proteins were eluted and subjected to electrophoresis and autoradiography. (**C**) T98G cells infected with a retrovirus expressing HA-tagged PDCD4 were deprived of serum (SD) for 72 hours and then activated with serum for 1 hour (SA). Cells were treated with MG132 for 3 hours before harvesting and lysis, and extracts were then subjected to immunoprecipitation with a HA-specific resin (anti-HA), followed by immunoblotting with antibodies to the indicated proteins. (**D**) T98G cells were infected with a retrovirus expressing either HA-tagged wild-type PDCD4 or HA-tagged PDCD4 mutants, as indicated. After serum deprivation (SD) for 72 hours, cells were activated with serum (SA) for the indicated times in the presence of cycloheximide (CHX). Cells were then collected, and proteins were analyzed by immunoblotting with an antibody against HA (left panels) to detect PDCD4 or with an antibody against CUL1 (right panels) to show normalization of the loading.

To gain insight into the pathways involved in the degradation of PDCD4, we reactivated mitogen-deprived cells with serum in the presence of various drugs. LY294002, a phosphoinositide 3-kinase (PI3K) inhibitor, prevented the proteolysis of PDCD4 (Fig. 1C). The fact that rapamycin, an inhibitor of mTOR (mammalian target of rapamycin), but not PI3K or the protein kinase AKT (which are both upstream to mTOR), had the same effect as LY294002 indicated that the mTOR-ribosomal protein S6 kinase 1 (S6K1) pathway is involved in promoting the degradation of PDCD4. The stabilization of PDCD4 paralleled (i) the lack of S6K1 phosphorylation and activation, (ii) the lack of S6 phosphorylation, and (iii) the decreased phosphorylation of 4EBP1, a known mTOR substrate. In contrast, the addition of SB203580 and U1026, inhibitors of p38 and extracellular signal–regulated kinases (ERKs), respectively, had no effect on the degradation of PDCD4.

The physical interaction between PDCD4 and βTRCP observed by mass spectrometry suggests that $SCF^{\beta TRCP}$ is the ubiquitin ligase targeting PDCD4 for degradation. This interaction is specific, because βTRCP1 and βTRCP2 were the only F-box proteins that coimmunoprecipitated with PDCD4 (fig. S2). Furthermore, PDCD4 contains a canonical βTRCP-binding motif [D^{70}SGRGDS^{76} (*10*) in human PDCD4] (fig. S3A). In all substrates investigated so far, the two serine residues in the DSGXX(X)S degron (where X represents any amino acid) must be phosphorylated to allow recognition by βTRCP (*11*). In many βTRCP substrates, phosphorylation on residues surrounding the degron promotes the phosphorylation of the two serine residues present in the degron (*1*). The putative βTRCP-binding motif in PDCD4 is immediately preceded by a Ser that is part of a canonical RXRXXS phosphorylation consensus site for S6K1 (R^{62}LRKNS^{67} in human PDCD4) (fig. S3B). We generated a number of PDCD4 mutants [all with hemagglutinin epitope (HA) tags] in which Ser^{67}, Ser^{71}, or Ser^{76} were mutated individually or in various combinations to Ala (e.g., Ser^{67} to Ala, S67A) (fig. S3C). After expression of these proteins in human embryonic kidney HEK293T cells, we immunoprecipitated them with HA-specific resin. Although wild-type PDCD4 immunoprecipitated efficiently with βTRCP1, the PDCD4(S71A), PDCD4(S76A), and PDCD4(S71/76A) mutants did not (Fig. 2A). This suggests that Ser^{71} and Ser^{76} may be phosphorylated in vivo and may mediate the binding to βTRCP. Accordingly, a peptide (amino acids 65 to 79) from PDCD4 containing both phosphorylated Ser^{71} and Ser^{76} efficiently bound βTRCP1 (but not FBXW5 and FBXW7), but a corresponding nonphosphorylated peptide did not (Fig. 2B).

Compared with the wild-type protein, PDCD4(S67A) displayed a markedly reduced binding (Fig. 2A). To investigate whether Ser^{67} is phosphorylated in cells, we used a phospho-specific antibody that recognizes the S6K1 phospho-consensus motif RXRXXpS (*12*). This antibody recognized wild-type PDCD4 but not PDCD4(S67A) or PDCD4(S67/71A) (Fig. 2A). Notably, PDCD4 appeared to be phosphorylated on Ser^{67} and associated with βTRCP1 in activated cells but not in serum-deprived cells (Fig. 2C).

Compared with wild-type PDCD4, the half-lives of PDCD4 mutants were increased in ac-

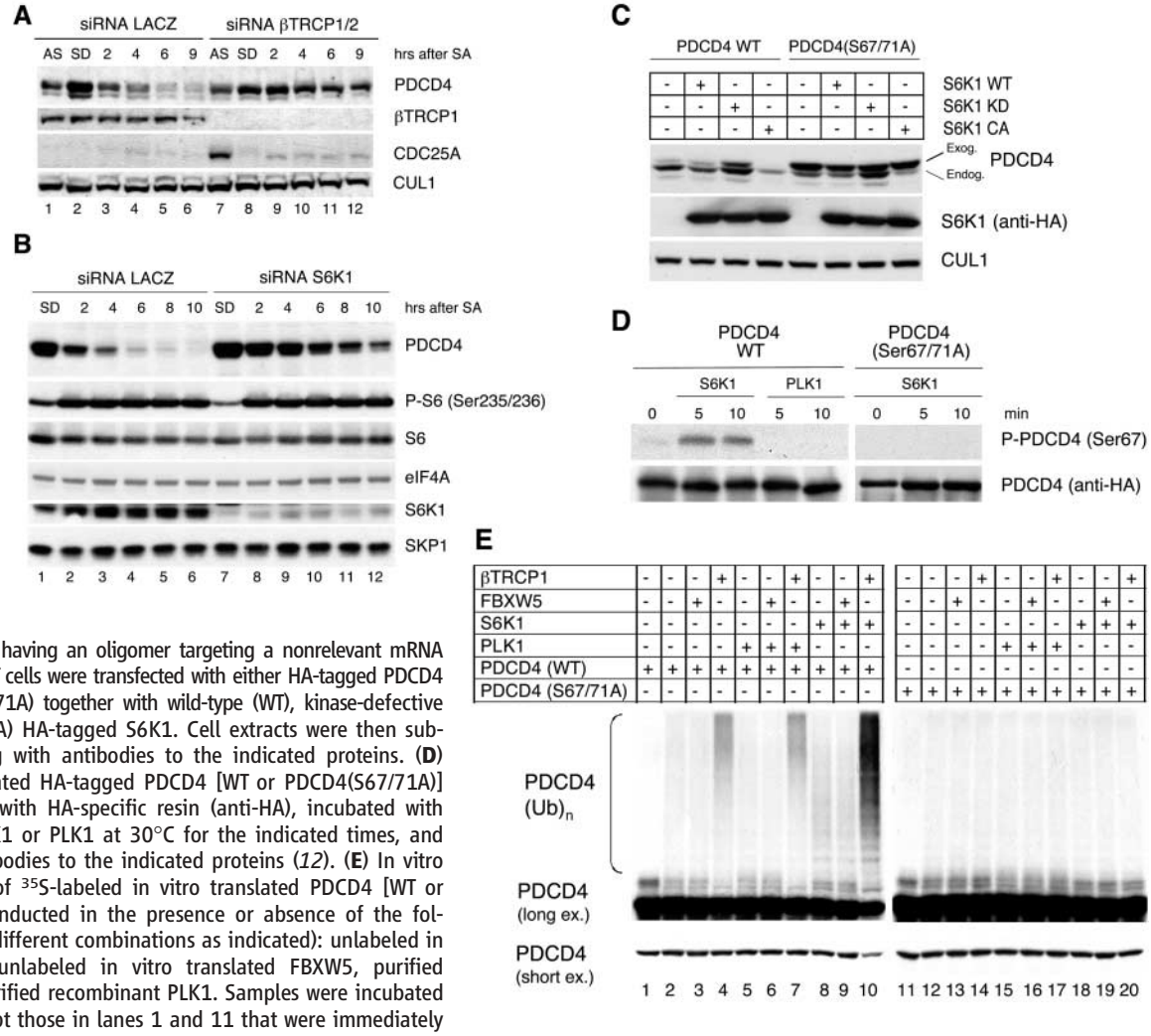


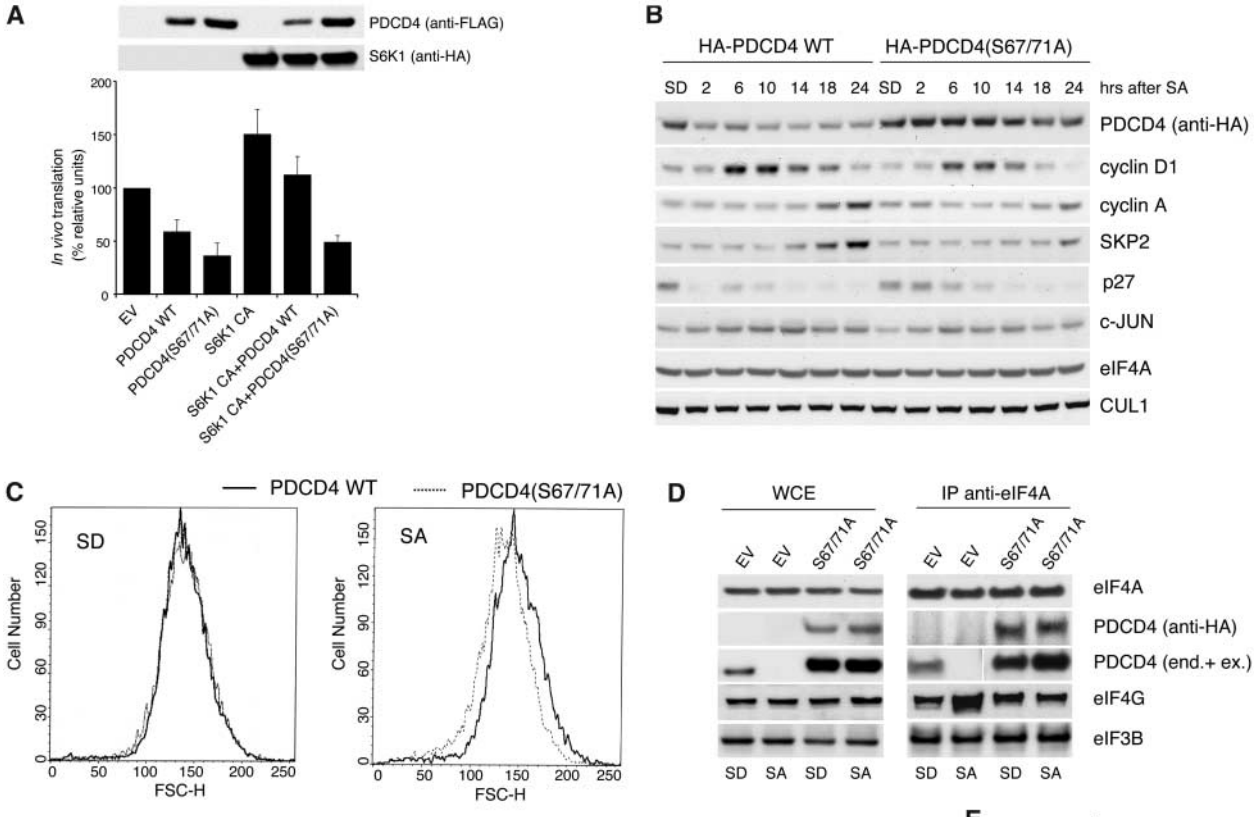
Fig. 3. Control of ubiquitylation and degradation of PDCD4 by βTRCP and S6K1. (**A**) T98G cells were transfected twice with siRNA molecules to a nonrelevant mRNA (LacZ) or to both βTRCP1 and βTRCP2 mRNA. Cells were deprived of serum (SD) for 36 hours and then activated with serum (SA) for the indicated hours. Lanes 1 and 7 show extracts from asynchronously growing cells (AS). Protein extracts were probed with antibodies to the indicated proteins. (**B**) The experiment was performed as in (A), except that effects with a dsRNA oligo to S6K1 (lanes 7 to 12) were compared with those having an oligomer targeting a nonrelevant mRNA (lanes 1 to 6). (**C**) HEK293T cells were transfected with either HA-tagged PDCD4 or HA-tagged PDCD4(S67/71A) together with wild-type (WT), kinase-defective T229A (KD), or T389E (CA) HA-tagged S6K1. Cell extracts were then subjected to immunoblotting with antibodies to the indicated proteins. (**D**) Unlabeled in vitro translated HA-tagged PDCD4 [WT or PDCD4(S67/71A)] was immunoprecipitated with HA-specific resin (anti-HA), incubated with purified recombinant S6K1 or PLK1 at 30°C for the indicated times, and immunoblotted with antibodies to the indicated proteins (*12*). (**E**) In vitro ubiquitin ligation assay of ^{35}S-labeled in vitro translated PDCD4 [WT or PDCD4(S67/71A)] was conducted in the presence or absence of the following proteins (used in different combinations as indicated): unlabeled in vitro translated βTRCP, unlabeled in vitro translated FBXW5, purified recombinant S6K1, or purified recombinant PLK1. Samples were incubated at 30°C for 90 min, except those in lanes 1 and 11 that were immediately added to sample buffer. The bracket on the left side of the top panels marks a ladder of bands corresponding to polyubiquitylated PDCD4.

tivated T98G cells (Fig. 2D) and correlated with their decreased binding to βTRCP (Fig. 2A). To further test whether βTRCP might regulate the stability of PDCD4, we used small interfering RNA (siRNA) to reduce the expression of βTRCP in T98G cells. We used a double-stranded RNA (dsRNA) oligomer that efficiently targets both βTRCP1 and βTRCP2 (*3*). Depletion of βTRCP inhibited the degradation of PDCD4 after serum addition (Fig. 3A).

Because Ser^{67} is part of a canonical phosphorylation site for S6K1 and because rapamycin and LY294002 inhibit PDCD4 degradation, we tested whether S6K1 might promote the βTRCP-mediated proteolysis of PDCD4. Inhibition of expression of S6K1 in cultured cells with a dsRNA oligomer (*13*) inhibited the phosphorylation (fig. S4) and degradation (Fig. 3B) of PDCD4. We also examined the effects of forced S6K1 expression on PDCD4 stability in HEK293 cells (Fig. 3C). Expression of wild-type S6K1 slightly reduced the amounts of endogenous and exogenous PDCD4, whereas the constitutively active (CA) S6K1 mutant (T389E) had a stronger effect. A kinase-defective (KD) S6K1 mutant (T229A) increased the abundance of PDCD4. Overexpression of wild-type S6K1 or S6K1 mutants had no effect on PDCD4(S67/71A) abundance, further corroborating the importance of these two residues in PDCD4 stability. The phosphorylation of PDCD4 on Ser^{67} by S6K1 was confirmed in a kinase assay using purified proteins (Fig. 3D). We propose that, in response to mitogens, S6K1 is activated and phosphorylates PDCD4 on Ser^{67}. This event, in turn, promotes the phosphorylation of Ser^{71} and Ser^{76} (by S6K1 or another kinase) and allows binding to βTRCP.

We reconstituted the ubiquitylation of PDCD4 in vitro. Wild-type PDCD4, but not the PDCD4(S67/71A) mutant, was efficiently ubiquitylated only when both βTRCP1 and S6K1 were present in the reaction mix (Fig. 3E and fig. S5). A different F-box protein, FBXW5, or a different kinase, PLK1, were unable to trigger the ubiquitylation of PDCD4 (Fig. 3E).

PDCD4 inhibits the cap-dependent translation of a luciferase mRNA with a stem-loop structured 5′UTR (pCMV-SL-LUC) more effi-



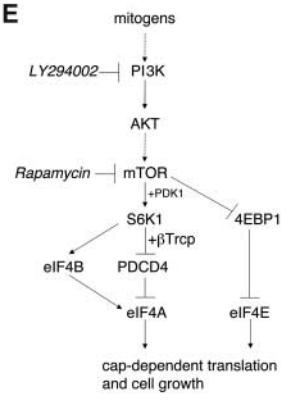


Fig. 4. Requirement of PDCD4 degradation for efficient protein translation, cell growth, and cell cycle progression. (**A**) T98G cells were transfected with the pCMV-SL-LUC reporter plasmid together with a pRL-null *Renilla* luciferase plasmid (for normalization) and constructs for the indicated proteins. Cells were allowed to recover for 18 hours and then were serum starved. Twenty-four hours later, cells were restimulated with serum for an additional 20 hours. Luciferase activities were measured by a dual-luciferase assay, and the luciferase/renilla light-unit ratio was calculated. The value of the sample transfected with an empty vector (EV) was set at 100%. Data are presented as the means ± SEM of three independent experiments (in triplicate). Amounts of exogenously expressed proteins (analyzed by immunoblotting with the indicated antibodies) are shown in the upper panels. (**B**) T98G cells were infected with a retrovirus expressing green fluorescent protein (GFP) and HA-tagged wild-type PDCD4 or HA-tagged PDCD4(S67/71A). Cells expressing low levels of PDCD4 (only about three times endogenous PDCD4) were isolated by fluorescence-activated cell sorting (FACS) (using GFP as a marker). After 72 hours of serum deprivation (SD), cells were activated with serum (SA) for the indicated times. Cells were harvested and analyzed by immunoblotting. (**C**) A representative experiment performed as in (B). Cell size was determined by FACS (forward scatter) in cells deprived of serum (SD) and 12 hours after serum addition (SA). (**D**) The experiment was performed as in (B). Four hours after serum addition (SA), whole-cell extracts (WCE) were either subjected directly to immunoblotting or to immunoprecipitation (IP) with an antibody against eIF4A, followed by immunoblotting with antibodies to the indicated proteins. (**E**) Model of how βTRCP and S6K1 control protein translation and cell size by promoting the degradation of PDCD4. For general reviews about protein translation, see references (*14*–*16*).

ciently than a nonstructured luciferase reporter (*8*). To study the biological significance of PDCD4 proteolysis, PDCD4 (wild-type or the S67/71A mutant) and CA S6K1 expression plasmids, as well as the pCMV-SL-LUC plasmid, were transiently transfected into T98G cells in various combinations, and then luciferase activities were measured (Fig. 4A). PDCD4(S67/71A) inhibited translation more efficiently than wild-type PDCD4, in agreement with its higher expression level after serum addition. Accordingly, silencing of βTRCP inhibited luciferase activity (fig. S6). In contrast, expression of CA S6K1 increased translation (Fig. 4A). Whereas wild-type PDCD4 poorly counteracted the effect of CA S6K1, expression of PDCD4(S67/71A) completely neutralized the stimulation by CA S6K1. All together, these results show that the regulated degradation of PDCD4 positively modulates protein translation in vivo.

We also infected T98G cells with retroviruses expressing HA-tagged wild-type PDCD4 or HA-tagged PDCD4(S67/71A). Cells were deprived of serum for 72 hours and then restimulated for various times (Fig. 4B). Expression of PDCD4(S67/71A) induced a slower accumulation of cyclin D1, cyclin A, and SKP2, and a slower degradation of p27. Thus, expression of PDCD4(S67/71A) delays the G_1-to-S phase transition of the cell cycle. Because S6K1 regulates cell growth (*14*, *15*), we measured cell size by flow cytometry. After serum stimulation, cells expressing PDCD4(S67/71A) displayed decreased cell size relative to cells expressing wild-type PDCD4 (Fig. 4C).

Finally, we investigated how the presence of larger amounts of PDCD4 influences the binding between eIF4A and eIF4G. In response to serum, when PDCD4 was degraded, the association between eIF4A and eIF4G increased more than twofold (Fig. 4D). However, in cells expressing PDCD4(S67/71A), this increase was no longer evident, correlating with the observed >50% inhibition of translation (Fig. 4A).

We propose that the degradation of PDCD4 in mitogen-stimulated cells is necessary for efficient protein translation, which is a prerequisite for cell growth and, consequently, cell proliferation (*16*, *17*) (Fig. 4E). Moreover, our findings implicate S6K1 and $SCF^{\beta TRCP}$ in the regulation of PDCD4 degradation and highlight the importance of the ubiquitin system in the control of translation initiation in response to mitogens.

References and Notes

1. D. Guardavaccaro, M. Pagano, *Mol. Cell* **22**, 1 (2006).
2. J. Jin *et al.*, *Genes Dev.* **18**, 2573 (2004).
3. A. Peschiaroli *et al.*, *Mol. Cell* **23**, 319 (2006).
4. A. Palamarchuk *et al.*, *Cancer Res.* **65**, 11282 (2005).
5. A. P. Jansen, C. E. Camalier, N. H. Colburn, *Cancer Res.* **65**, 6034 (2005).
6. A. Goke *et al.*, *Biochem. Biophys. Res. Commun.* **297**, 78 (2002).
7. H. S. Yang *et al.*, *Mol. Cell. Biol.* **23**, 26 (2003).
8. H. S. Yang *et al.*, *Mol. Cell. Biol.* **24**, 3894 (2004).
9. M. J. Kang *et al.*, *Biochem. Biophys. Res. Commun.* **293**, 617 (2002).
10. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr; and X, any amino acid.
11. T. Cardozo, M. Pagano, *Nat. Rev. Mol. Cell Biol.* **5**, 739 (2004).
12. H. Zhang *et al.*, *J. Biol. Chem.* **277**, 39379 (2002).
13. M. K. Holz, J. Blenis, *J. Biol. Chem.* **280**, 26089 (2005).
14. G. Thomas, *Biol. Res.* **35**, 305 (2002).
15. A. F. Shamji, P. Nghiem, S. L. Schreiber, *Mol. Cell* **12**, 271 (2003).
16. N. Hay, N. Sonenberg, *Genes Dev.* **18**, 1926 (2004).
17. D. C. Fingar, J. Blenis, *Oncogene* **23**, 3151 (2004).

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Supporting Online Material

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References

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Functional Delivery of a Cytosolic tRNA into Mutant Mitochondria of Human Cells

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Many maternally inherited and incurable neuromyopathies are caused by mutations in mitochondrial (mt) transfer RNA (tRNA) genes. Kinetoplastid protozoa, including *Leishmania*, have evolved specialized systems for importing nucleus-encoded tRNAs into mitochondria. We found that the *Leishmania* RNA import complex (RIC) could enter human cells by a caveolin-1–dependent pathway, where it induced import of endogenous cytosolic tRNAs, including $tRNA^{Lys}$, and restored mitochondrial function in a cybrid harboring a mutant mt $tRNA^{Lys}$ (*MT-TK*) gene. The use of protein complexes to modulate mitochondrial function may help in the management of such genetic disorders.

The A8344G mutation (A to G nucleotide mutation at position 8344) in the *MT-TK* gene in myoclonic epilepsy with ragged red fibers (MERFF) (*1*) causes a substantial reduction in the rate of translation of most mitochondrial mRNAs, as well as the accumulation of aberrant translation products (*2*), which results from inefficient aminoacylation of (*3*) and/or codon recognition by (*4*) the mutant tRNA. More severe defects are observed in patients possessing deletions of this gene: for example, in Kearns-Sayre syndrome (KSS), a 1.9-kb mitochondrial deletion covers the *MT-TK* gene, as well as the neighboring *CO2*, *CO3*, *ATP6*, and *ATP8* genes (*5*). Cytoplasmic hybrids (cybrids), derived from such patients and carrying mutant mitochondria, are good in vitro models for monitoring the efficacy of correctional protocols. Partial rescue of mitochondrial function in cybrids, and in primary fibroblasts from MERRF patients, by transfection of variants of yeast $tRNA^{Lys}$ genes has been reported (*6*), but this approach suffers from low efficiency, variability, and toxicity of the transfection vehicle.

The RNA import complex (RIC), isolated from the inner membrane of *Leishmania* mitochondria, is a large (M_r ~ 600 kD) multisubunit aggregate containing several tRNA binding proteins (fig. S1 and table S2) that is active for specific, adenosine triphosphate (ATP)–dependent translocation of tRNAs (*7*, *11*). Human cytoplasmic $tRNA^{Lys}$(UUU) is imported into the mitochondria of transgenic *Trypanosoma brucei* (*8*) and transiently transfected *L. tropica* (*9*), as well as into isolated *Leishmania* mitochondria (*9*). Moreover, purified RIC induces import of the same tRNA into human mitochondria in vitro (*10*). The imported tRNA undergoes multiple rounds of lysylation within the organelle and directly donates lysine to the translating ribosome, correcting the translational defects in MERRF-derived mutant mitochondria (*10*). We tested the hypothesis that RIC can be used to induce functional tRNA import in whole cells.

To monitor uptake of RIC, we incubated monolayer cultures of either the human hepatocarcinoma (Hep) G2 cell line or cybrids containing wild-type (LB58), MERRF patient–derived (LB64), or KSS (FLP32.39) mitochondria with affinity-purified fluorescent-tagged RIC (*11*), and live cells were imaged at various

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S6K1- and ßTRCP-Mediated Degradation of PDCD4 Promotes Protein Translation and Cell Growth

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