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SHORT COMMUNICATION

The autophagic inducer smARF interacts with and is stabilized by the mitochondrial p32 protein

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The alternative reading frame (ARF) mRNA encodes two pro-death proteins, the nucleolar p19ARF and a shorter mitochondrial isoform, named smARF (hsmARF in human). While p19ARF can inhibit cell growth by causing cell cycle arrest or type I apoptotic cell death, smARF is able to induce type II autophagic cell death. Inappropriate proliferative signals generated by proto-oncogenes, such as c-Myc and E2F1, can elevate both p19ARF and smARF proteins. Here, we reveal a novel means of regulation of smARF protein steady state levels through its interactions with the mitochondrial p32. The p32 protein physically interacts with both human and murine smARF, and colocalizes with these short isoforms to the mitochondria. Remarkably, knocking down p32 protein levels significantly reduced the steady state levels of smARF by increasing its turn over. As a consequence, the ability of ectopically expressed smARF to induce autophagy and to cause mitochondrial membrane dissipation was significantly reduced. In contrast, the protein levels of full-length p19ARF, which mainly resides in the nucleolus, were not influenced by p32 depletion, suggesting that p32 exclusively stabilizes the mitochondrial smARF protein. Thus the interaction with p32 provides a means of specifically regulating the expression of the recently identified autophagic inducer, smARF, and adds yet another layer of complexity to the multifaceted regulation of the ARF gene.
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The mouse p19 alternative reading frame (ARF) tumor suppressor (p14ARF in human) is localized to the nucleolus, and can inhibit cell growth in a p53-dependent or -independent manner (Sherr, 2006). Recently, we found that ARF mRNA also encodes an additional shorter mitochondrial isoform, named smARF (hsmARF in human), that is translated from an internal methionine at position 45 and therefore lacks all the nucleolar functions of the full-length protein. smARF is a short-lived protein that is rapidly degraded by the proteasome, but accumulates after inappropriate proliferative signals generated by oncogenes. Overexpression of this isoform results in damage to the structure of the mitochondria, dissipation of the mitochondrial membrane potential and autophagic cell death (Reef *et al*., 2006). A search for novel p19ARF interacting proteins, based on immunopurification of ectopically expressed p19ARF from total cell lysates and identification of co-immunoprecipitated bands by mass spectrometry, yielded several candidate proteins. One of them was the p32 protein (Figure 1a). The p32 protein (named also gC1qR, SF2-associated binding protein) is a doughnut-shaped homotrimeric protein (Jiang *et al*., 1999), which localizes predominantly to the mitochondrial matrix (Muta *et al*., 1997; Dedio *et al*., 1998; Matthews and Russell, 1998; Seytter *et al*., 1998). p32 has been shown to bind many cellular (Ghebrehiwet *et al*., 1994; Simos and Georgatos, 1994; Yu *et al*., 1995a; Deb and Datta, 1996; Herwald *et al*., 1996; Lim *et al*., 1996) and viral proteins (Desai *et al*., 1991; Luo *et al*., 1994; Fridell *et al*., 1995; Yu *et al*., 1995b; Bruni and Roizman, 1996; Tange *et al*., 1996; Wang *et al*., 1997; Matthews and Russell, 1998). Despite abundant biochemical data, many aspects in p32s function are still unknown, although it has been suggested that it has a role in the maintenance of mitochondrial oxidative phosphorylation in yeast (Muta *et al*., 1997), and that it can regulate Hrk-mediated apoptosis in mammalian cells (Sunayama *et al*., 2004). We found that the human p14ARF was also capable to pull down the p32 protein (data not shown). Moreover, the interaction between ARF protein and p32 was direct, since ^{35}S-labeled p32, produced by *in vitro* translation in rabbit reticulocyte lysate, was pulled-down by glutathione *S*-transferase (GST)-p14ARF, but not by GST alone, GST-Hdm2 or GST-p53 (Figure 1b). Thus, although the mouse and human ARF proteins share a relatively low degree of identity over the region of overlap (45%), the ability to bind p32 is conserved, implying the significance of this interaction.

Since p32 is a mitochondrial protein, it was appealing to examine whether smARF, the mitochondrial ARF short isoform, can also interact with p32. To this end, 293T cells were transfected with Flag-tagged smARF

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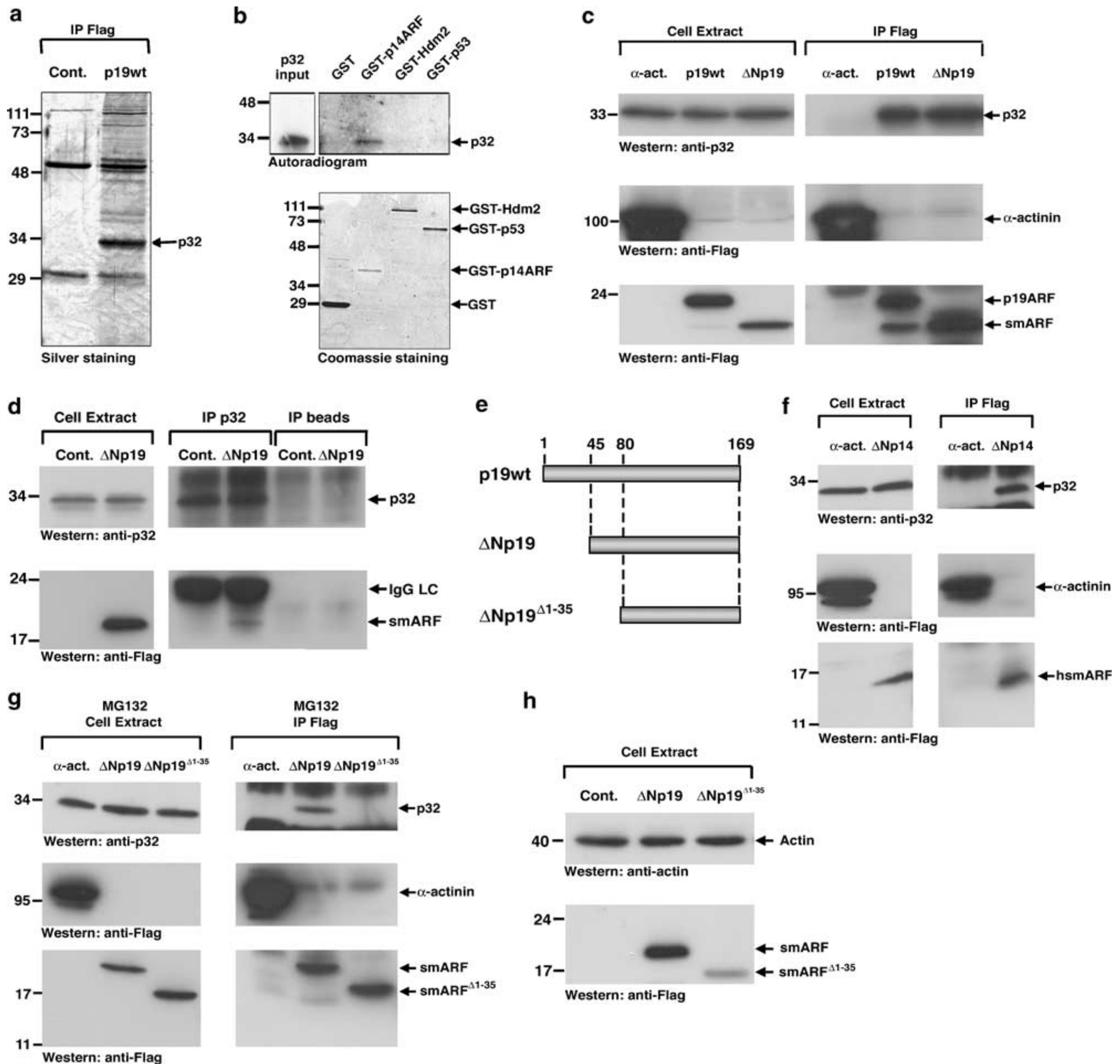

Figure 1 The mitochondrial ARF isoform interacts with p32. (**a**) 293T cells were transfected with either Flag tagged p19ARF (p19wt) or empty control vector (Cont.). After 48 h, the cells were harvested and lysed in NP40 buffer (150 mM NaCl, 50 mM Tris, pH 8.0 and 1% NP40). The extracts were immunoprecipitated with anti-Flag antibodies (Sigma, Rehovot, IL, USA) prebound to protein A-sepharose beads and washed with NP40 buffer, followed by elution with excess free Flag epitope peptide. Eluates were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), followed by silver staining. The position of the p32 band, identified by mass spectrometry, is indicated. (**b**) p32 was produced by *in vitro* translation in rabbit reticulocyte lysate in the presence of ^{35}S-Met. Equal amounts of radiolabeled p32 were incubated with the indicated GST fusion proteins or with GST alone. Bound proteins were eluted, and resolved by SDS–PAGE and visualized by exposure to X-ray film. The GST fusion proteins were visualized by Coomassie staining. (**c**) Flag-tagged smARF (ΔNp19) and full-length p19ARF were transiently transfected into 293T cells and immunopurified with anti-Flag antibodies. Shown are western blots of total cell lysates (left) and of the specific anti-Flag immunoprecipitates (right), probed with mouse anti-Flag (Sigma) and rabbit anti-p32 antibodies (a generous gift from WC Russell (Matthews and Russell, 1998)). Flag-tagged α-actinin was used as a negative control. (**d**) Flag-tagged smARF (ΔNp19) and luciferase (Cont.) were transiently transfected into 293T cells and immunopurified with mouse anti-p32 antibodies (Santa Cruz, Santa Cruz, CA, USA). Shown are western blots of total cell lysates (left) and of the specific anti-p32 immunoprecipitates (right), probed with mouse anti-Flag (Sigma) and rabbit anti-p32 antibodies. Empty beads were used as a negative control. (**e**) Schematic presentation of full-length p19ARF (p19wt), smARF (ΔNp19) and N-terminal truncated smARF ($\Delta Np19^{\Delta 1-35}$) proteins. (**f**) Flag-tagged hsmARF (ΔNp14) were transiently transfected into 293 T cells and immunopurified with anti-Flag antibodies. Flag-tagged α-actinin was used as a negative control. The total cell lysates and immunoprecipitates were processed like in (**c**). (**g**) Flag-tagged smARF and the N-terminal truncated smARF mutant ($\Delta Np19^{\Delta 1-35}$) were transfected into 293T cells treated with the MG132 (Calbiochem, San Diego, CA, USA; 10 μM, 17 h), the total cell lysates and immunoprecipitates were processed like in (**c**). (**h**) The indicated plasmids were transiently transfected into 293 T cells. Western blot analysis was performed 24 h later, with anti-Flag or anti-actin antibodies (Sigma).

(ΔNp19- see the scheme in Figure 1e) or hsmARF (ΔNp14- the corresponding human protein lacking the first 47 amino acids). Flag-tagged α-actinin was used as a non-relevant negative control. The proteins were immunoprecipitated using anti-Flag antibodies. Flag-tagged smARF pulled down endogenous p32, similar to the full-length p19ARF, while Flag-tagged α-actinin could not (Figure 1c). Similarly, in the reciprocal immunoprecipitation, endogenous p32 specifically pulled down Flag-smARF (Figure 1d). Owing to the low expression levels of endogenous smARF, and the background appearing at the expected size with the available anti-p19ARF antibodies, we were not able to detect specific co-IP with the endogenous smARF. In addition, similar to smARF, Flag-tagged hsmARF also co-immunoprecipitated with p32 (Figure 1f). The fact that both smARF and hsmARF are able to interact with p32 indicates that p19/p14ARFs N terminus (aa 1–44, or 1–47, respectively), which drives the nucleolar functions of the full-length protein, is not essential for the interaction with p32. Notably, further truncation of a stretch of 35 amino acids from the N-terminal region of smARF (ΔNp19$^{\Delta 1-35}$ see the scheme in Figure 1e) abrogated the binding to p32 (Figure 1g), suggesting, that the first 35 amino acids of smARF are necessary for the interaction. The N-terminal truncation of smARF also caused further destabilization of the protein (Figure 1h), and therefore, to reach comparable levels of the two proteins, the pull down experiment in Figure 1g was carried out in the presence of the proteasome inhibitor MG132.

The p32 protein was shown to be expressed mainly in the soluble matrix of the mitochondria (Muta *et al*., 1997; Dedio *et al*., 1998; Matthews and Russell, 1998; Seytter *et al*., 1998). However, several studies have reported that under certain conditions, a small fraction of p32 can be found also in other cellular compartments, such as the nucleus and the plasma membrane (Matthews and Russell, 1998; Soltys *et al*., 2000; Brokstad *et al*., 2001). Therefore, the localization of p32 was examined in HeLa cells by staining with specific anti-p32 antibodies and compared by co-staining to Flag-tagged smARF or hsmARF. In all of the smARF/hsmARF transfected cells, endogenous p32 was found colocalized with the short ARF isoform to punctate structures (Figure 2a, rows I and III). These structures stain positively for the mitochondrial marker cytochrome *c* (Figure 2a, row II), and are in fact the fragmented, damaged mitochondria generated by smARF expression (Reef *et al*., 2006). Unlike the smARF/hsmARF-expressing cells, the adjacent non-transfected cells when stained with anti-p32 antibodies, always showed the typical tubular staining of intact mitochondria (Figure 2a, row I and III), which overlapped with the cytochrome *c* staining (Figure 2a, row II). The colocalization of p32 and smARF to the mitochondria was also confirmed in 293T cells (data not shown). Notably, in a fraction of cells expressing ectopic smARF or hsmARF, nucleolar staining was also observed. However, even in those cells, endogenous p32 was detected only in the mitochondria, and not in the nucleolus (data not shown), indicating that the ARF short isoform interacts with p32 in the mitochondria and not in the nucleolus. Of note, the truncated mutant ΔNp19$^{\Delta 1-35}$, which could not interact efficiently with

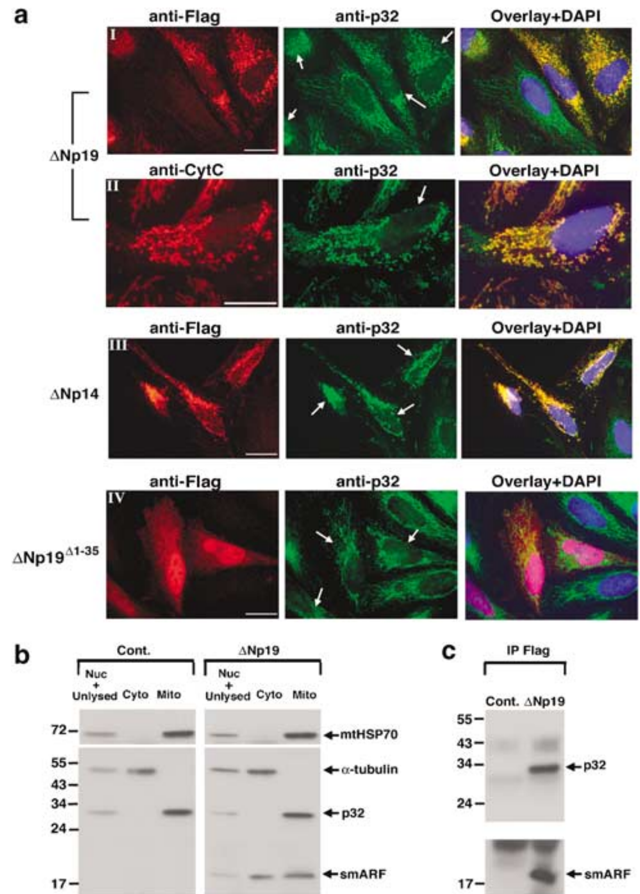

Figure 2 smARF and hsmARF colocalize with p32 to the mitochondria. (**a**) HeLa cells grown on glass coverslips were transiently transfected with ΔNp19. After 24 h the cells were fixed with 3.7% formaldehyde, and then permeabilized/blocked with 0.4% Triton-X 100 (Sigma) in 10% normal goat serum (Biological Industries, Beit Haemek, IL, USA), and incubated with mouse anti-Flag (row I) or anti-cytochrome C antibodies (Santa Cruz) (row II) and with rabbit anti-p32 antibodies, followed by Cy2-conjugated donkey anti-rabbit secondary antibodies and rhodamine-conjugated goat anti-mouse secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA). Similar transfections with ΔNp14 or ΔNp19$^{\Delta 1-35}$ and staining with mouse anti-Flag and rabbit anti-p32 antibodies are shown in rows III and IV, respectively. The arrows point to smARF/hsmARF/smARF$^{\Delta 1-35}$ transfected cells. Cells were finally stained with DAPI (0.5 μg/ml, Sigma) and examined by fluorescent light microscopy (Olympus BX41) with $\times 60$ (NA 1.25) or 100 (NA 1.3) UPlan-Fl oil immersion objectives. Bar represents 5 μm. (**b**) Cellular extracts from 293T cells transfected with ΔNp19ARF or luciferase (Cont.) were fractionated into nuclear, cytosolic and mitochondrial fractions as described before (Reef *et al*., 2006; 25 μg of each fraction per lane). Note that the nuclear fraction was contaminated with unlysed cells. To assess the purity of the fractions, mtHSP70 and α-tubulin were used as markers for the mitochondria and cytoplasm, respectively. (**c**) The enriched mitochondrial fraction purified from cells expressing Flag-tagged smARF (ΔNp19) or luciferase (Cont.), was subjected to immunopurification with anti-Flag antibodies. Shown are western blots of the specific anti-Flag immunoprecipitates, probed with anti-Flag (Sigma) and rabbit anti-p32 antibodies.

p32, was no longer exclusively mitochondrial, but could be found also in the cytoplasm and/or the nucleus (Figure 2a, row IV). Thus the degree of co-immunostaining of p32 and the truncated mutant was severely reduced. This suggests that the first 35 amino acids of smARF are also necessary for its appropriate mitochondrial localization. To confirm further the mitochondrial colocalization of smARF and p32, the mitochondria were fractionated from 293T cells which were transfected with ΔNp19ARF (smARF) or luciferase (Cont.) constructs. The purified mitochondrial fraction clearly contained both p32 and smARF proteins (Figure 2b). Additionally, p32 co-immunoprecipitated with Flag-tagged smARF specifically from the mitochondrial

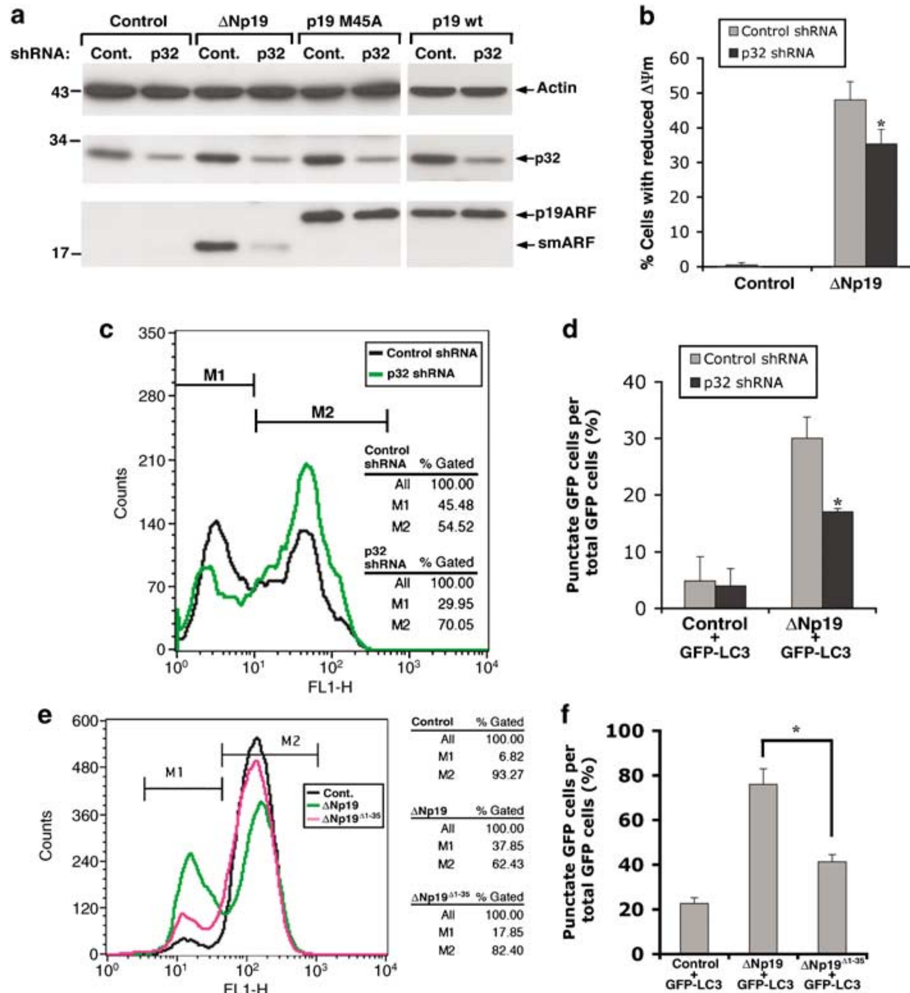

Figure 3 Knockdown of endogenous p32 affects the steady state levels of smARF. (**a**) 293T cells were transfected with HcRed (control) (targeting nucleotides 99–117 of HcRed (Accession number AF363776)) or p32 shRNA plasmids (targeting nucleotides 540–558 of p32 (accession number NM_001212; Sunayama *et al*., 2004)). After 4 days, the cells were re-transfected with luciferase (control), 1 μg ΔNp19ARF, 0.25 μg p19ARF M45A or 1 μg of p19ARF plasmids. Western blot analysis was preformed 24 h later, with rabbit anti-p32 antibodies, and mouse anti-actin or anti-Flag antibodies. (**b**) 293T cells were transfected with HcRed or p32 shRNA plasmids. After 4 days, the cells were co-transfected with 1 μg GFP and 10 μg luciferase (control) or 2 μg ΔNp19ARF and 8 μg luciferase plasmids. 24 h later, the cells were incubated for 30 min with 100 nM MitoTracker dye (CMX-Ros; Molecular Probes, Carlsbad, CA, USA) at 37°C. The graph represents quantitation of cells exhibiting reduced mitochondrial membrane potential. Data presented is the mean ± s.d. calculated from triplicates of 100 transfected cells each. *denotes $P = 0.031$. (**c**) 293T cells were transfected with HcRed or p32 shRNA plasmids. After 4 days, the cells were co-transfected with either 10 μg luciferase (control) or 1 μg ΔNp19ARF and 9 μg luciferase plasmids. 24 h later, the cells were assayed for loss of mitochondrial membrane potential by flow cytometric analysis of $DiOC_6$ fluorescence. Histograms represent cell counts versus fluorescence intensity. Two distinct populations of cells were defined by their levels of fluorescent intensities: M1-cells with reduction in $\Delta\Psi_m$, M2-cells with intact $\Delta\Psi_m$. (**d**) 293T cells were transfected with HcRed or p32 shRNA plasmids. After 4 days, the cells were co-transfected with 0.5 μg GFP-LC3 and either 10 μg luciferase or 2 μg ΔNp19ARF and 8 μg luciferase. 24 h later, the cells with punctate GFP-LC3 were quantified per total GFP-LC3-positive cells. Data represent mean ± s.d. calculated from triplicates of 100 transfected cells each. *$P < 0.005$. (**e**) NIH3T3 cells expressing 10 μg luciferase (Cont.) (black), smARF (ΔNp19ARF) (green) or smARF$^{\Delta 1-35}$ (ΔNp19ARF$^{\Delta 1-35}$) (pink) were assayed for loss of mitochondrial membrane potential 24 h after transfection by flow cytometric analysis of $DiOC_6$ fluorescence as described in (c). (**f**) 293T cells were co-transfected with 1 μg GFP-LC3 and either 10 μg luciferase, ΔNp19ARF or ΔNp19ARF$^{\Delta 1-35}$. After 72 h, the cells with punctate GFP-LC3 were quantified per total GFP-LC3-positive cells. Data presented is the mean ± s.d. calculated from triplicates of 100 transfected cells each. *$P < 0.002$. Statistical analysis shown in (**b**, **d** and **f**) was performed by a two-tailed Student's *t*-test.

fraction (Figure 2c). Thus, the co-immunostaining of smARF and hsmARF with the mitochondrial p32, together with the physical interaction between smARF and p32 when extracted from purified mitochondria, suggest that the mitochondrial compartment is the main cellular milieu where smARF and hsmARF interact with p32 within cells. We therefore assume that the binding of the full-length nucleolar p19ARF to p32 shown in Figure 1a and c probably occurred after cell lysis in the crude extracts.

To examine whether smARFs function is dependent on p32, an shRNA plasmid was generated to knock down endogenous p32. The levels of p32 protein were partially reduced upon transfection of 293T cells with the shRNA construct (Figure 3a). Remarkably, the knock down of p32 significantly reduced the steady-state levels of ectopically expressed smARF when the latter was expressed below saturation levels. In contrast, the steady-state levels of full-length p19ARF were not affected upon similar transfection with p19M45A (mutated at Met45, which initiates smARF expression (Reef *et al*., 2006)), or with the wild type construct, which express mainly the full-length p19ARF (Figure 3a). Thus, p32 is capable of regulating smARFs protein expression levels, and does not affect the steady state levels of the full-length p19ARF. Next, the effect of p32 depletion on the previously reported smARF-induced dissipation of the mitochondrial membrane potential was tested. To this end, 293T cells in which p32 had been knocked down, were transfected with control or ΔNp19ARF expression vectors. After 24 h, the transfected cells were stained with a fluorescent mitochondrial dye, MitoTracker Red, which accumulates only in actively respiring mitochondria that have an intact mitochondrial membrane potential. The cells were then fixed and immunostained with anti-Flag antibodies. Expression of smARF in the p32 knock down cells led to smaller reductions in the mitochondrial membrane potential compared to cells transfected with control shRNA (Figure 3b), as would be expected considering that lower levels of smARF are expressed in these cells. Similar result was obtained by using flow cytometric analysis of transfected cells stained with the mitochondrial probe $DiOC_6$. The loss of mitochondrial membrane potential caused by smARF was attenuated from 45.5% in cells expressing the normal levels of p32 (control shRNA), to only 30% in p32 knock down cells (Figure 3c). The final cellular outcome of smARF overexpression, which involves induction of autophagy, was also examined after p32 depletion. To that end, 293T cells in which p32 had been knocked down were co-transfected with green fluorescent protein (GFP)-LC3 and ΔNp19ARF. The extent of autophagy induction, that is, of smARF-transfected cells displaying punctate LC3 staining, was reduced by knocking down p32 (Figure 3d). As expected, the truncated mutant $\Delta Np19^{\Delta 1-35}$, which could not interact efficiently with p32, and which was not exclusively mitochondrial, and expressed to lower levels, was less efficient both in reducing the mitochondrial membrane potential (Figure 3e), and in inducing autophagic vesicle formation (Figure 3f). Notably, the effect of p32 on smARF was concentration-dependent; above a certain threshold of smARF expression, p32 levels no longer influenced its steady-state levels, and therefore, knock down of p32 did not affect smARFs ability to dissipate membrane potential or induce autophagy (data not shown). This implies that p32s direct role is to regulate the steady-state expression of smARF, rather than its function.

To examine the effect of p32 protein on smARF steady state levels under physiological conditions, 35-8 cells (immortalized p53-null MEFs) were electroporated with siRNA against murine p32, and the expression levels of p19ARF isoforms were examined. Notably, in cells in which p32 was knocked down, the expression levels of endogenous smARF were significantly reduced, but the expression levels of full-length p19ARF were not affected (Figure 4). To examine whether the reduction of smARF protein levels in p32 knockdown cells was due to differences in protein turnover, the stability of smARF protein following administration of cycloheximide was measured in the 35-8 cells. Notably, the

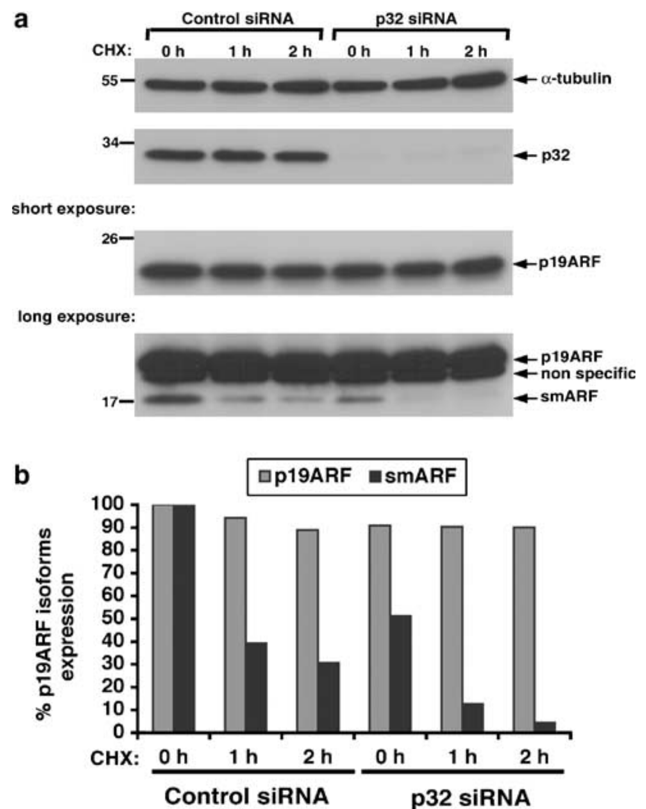

Figure 4 p32 protein stabilizes smARF under physiological conditions. (**a**) 35-8 immortalized MEFs that lack p53 were electroporated (Amaxa, Gaithersburg, MD, USA) with HcRed or p32 siRNA (Dharmacon, Chicago, IL, USA). After 48 h, the cells were replated and were treated with 20 μg/ml cycloheximide (Sigma) for the indicated times, or remained untreated. Western blot analysis on 12% gels was preformed with mouse anti-p32 (Santa Cruz) and anti-α-tubulin antibodies (Sigma), or rabbit anti-p19ARF antibodies (Abcam, Cambridge, MA, USA). (**b**) Quantitation of the expression levels of p19ARF isoforms shown in (**a**). Densitometry was calculated using the NIH Image program on scanned blots.

turnover of smARF in p32 knockdown cells was faster than in control cells (Figure 4), suggesting that depletion of p32 enhances the degradation rate of smARF. As mentioned previously, the N-terminal truncated mutant (ΔNp19$^{\Delta 1-35}$), which fails to bind to p32 (Figure 1g), was significantly more unstable than ΔNp19 (compare Figure 1h and g), consistent with the conclusion that p32 stabilizes smARF. Although p32 was also able to bind to ectopically expressed full-length p19ARF in cell lysates, it could not regulate its turnover, underscoring the specificity of the regulation of smARF by p32 protein and further suggesting that smARF is the ultimate physiological partner of p32 in intact cells. Thus, unlike oncogene expression, which enhances the expression of both isoforms (Reef *et al*., 2006), probably by common mechanisms which operate at the transcriptional level, different proteins control the turnover of the nucleolar and mitochondrial isoforms. p19ARF is stabilized through interaction with nucleolar NPM/B23 protein (Kuo *et al*., 2004), and smARF is specifically stabilized by the mitochondrial p32 protein. These findings reveal a novel function for p32, which, although shown to interact with many proteins, the full repertoire of its cellular functions was not ascribed yet. A future challenge will be to reveal the mechanism by which p32 regulates the stabilization of smARF. As previously suggested by using the MG132 inhibitor, smARF is kept under tight regulation by proteasome-mediated degradation (Reef *et al*., 2006). p32 may protect smARF from such degradation by binding it and sequestering it to the mitochondria. Alternatively, smARF degradation may occur within the mitochondria via ATP-dependent mitochondrial proteases that may be also sensitive to the proteasome inhibitor MG132 (Granot *et al*., 2003). In such a scenario, p32 may protect smARF from these proteases within the mitochondria. Additional experiments are required to gain further insight into this novel pathway.

In summary, the interaction with p32 provides a means of specifically regulating the expression of the recently identified autophagic inducer, smARF.

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References

Brokstad KA, Kalland KH, Russell WC, Matthews DA. (2001). Mitochondrial protein p32 can accumulate in the nucleus. *Biochem Biophys Res Commun* **281**: 1161–1169.

Bruni R, Roizman B. (1996). Open reading frame P – a herpes simplex virus gene repressed during productive infection encodes a protein that binds a splicing factor and reduces synthesis of viral proteins made from spliced mRNA. *Proc Natl Acad Sci USA* **93**: 10423–10427.

Deb TB, Datta K. (1996). Molecular cloning of human fibroblast hyaluronic acid-binding protein confirms its identity with P-32, a protein co-purified with splicing factor SF2. Hyaluronic acid-binding protein as P-32 protein, co-purified with splicing factor SF2. *J Biol Chem* **271**: 2206–2212.

Dedio J, Jahnen-Dechent W, Bachmann M, Muller-Esterl W. (1998). The multiligand-binding protein gC1qR, putative C1q receptor, is a mitochondrial protein. *J Immunol* **160**: 3534–3542.

Desai K, Loewenstein PM, Green M. (1991). Isolation of a cellular protein that binds to the human immunodeficiency virus Tat protein and can potentiate transactivation of the viral promoter. *Proc Natl Acad Sci USA* **88**: 8875–8879.

Fridell RA, Harding LS, Bogerd HP, Cullen BR. (1995). Identification of a novel human zinc finger protein that specifically interacts with the activation domain of lentiviral Tat proteins. *Virology* **209**: 347–357.

Ghebrehiwet B, Lim BL, Peerschke EI, Willis AC, Reid KB. (1994). Isolation, cDNA cloning, and overexpression of a 33-kD cell surface glycoprotein that binds to the globular 'heads' of C1q. *J Exp Med* **179**: 1809–1821.

Granot Z, Geiss-Friedlander R, Melamed-Book N, Eimerl S, Timberg R, Weiss AM *et al*. (2003). Proteolysis of normal and mutated steroidogenic acute regulatory proteins in the mitochondria: the fate of unwanted proteins. *Mol Endocrinol* **17**: 2461–2476.

Herwald H, Dedio J, Kellner R, Loos M, Muller-Esterl W. (1996). Isolation and characterization of the kininogen-binding protein p33 from endothelial cells. Identity with the gC1q receptor. *J Biol Chem* **271**: 13040–13047.

Jiang J, Zhang Y, Krainer AR, Xu RM. (1999). Crystal structure of human p32, a doughnut-shaped acidic mitochondrial matrix protein. *Proc Natl Acad Sci USA* **96**: 3572–3577.

Kuo ML, den Besten W, Bertwistle D, Roussel MF, Sherr CJ. (2004). N-terminal polyubiquitination and degradation of the ARF tumor suppressor. *Genes Dev* **18**: 1862–1874.

Lim BL, Reid KB, Ghebrehiwet B, Peerschke EI, Leigh LA, Preissner KT. (1996). The binding protein for globular heads of complement C1q, gC1qR. Functional expression and characterization as a novel vitronectin binding factor. *J Biol Chem* **271**: 26739–26744.

Luo Y, Yu H, Peterlin BM. (1994). Cellular protein modulates effects of human immunodeficiency virus type 1 Rev. *J Virol* **68**: 3850–3856.

Matthews DA, Russell WC. (1998). Adenovirus core protein V interacts with p32–a protein which is associated with both the mitochondria and the nucleus. *J Gen Virol* **79**(Part 7): 1677–1685.

Muta T, Kang D, Kitajima S, Fujiwara T, Hamasaki N. (1997). p32 protein, a splicing factor 2-associated protein, is localized in mitochondrial matrix and is functionally important in maintaining oxidative phosphorylation. *J Biol Chem* **272**: 24363–24370.

Reef S, Zalckvar E, Shifman O, Bialik S, Sabanay H, Oren M *et al*. (2006). A short mitochondrial form of p19ARF induces autophagy and caspase-independent cell death. *Mol Cell* **22**: 463–475.

Seytter T, Lottspeich F, Neupert W, Schwarz E. (1998). Mam33p, an oligomeric, acidic protein in the mitochondrial

matrix of Saccharomyces cerevisiae is related to the human complement receptor gC1q-R. *Yeast* **14**: 303–310.

Sherr CJ. (2006). Divorcing ARF and p53: an unsettled case. *Nat Rev Cancer* **6**: 663–673.

Simos G, Georgatos SD. (1994). The lamin B receptor-associated protein p34 shares sequence homology and antigenic determinants with the splicing factor 2-associated protein p32. *FEBS Lett* **346**: 225–228.

Soltys BJ, Kang D, Gupta RS. (2000). Localization of P32 protein (gC1q-R) in mitochondria and at specific extra-mitochondrial locations in normal tissues. *Histochem Cell Biol* **114**: 245–255.

Sunayama J, Ando Y, Itoh N, Tomiyama A, Sakurada K, Sugiyama A. (2004). Physical and functional interaction between BH3-only protein Hrk and mitochondrial pore-forming protein p32. *Cell Death Differ* **11**: 771–781.

Tange TO, Jensen TH, Kjems J. (1996). *In vitro* interaction between human immunodeficiency virus type 1 Rev protein and splicing factor ASF/SF2-associated protein, p32. *J Biol Chem* **271**: 10066–10072.

Wang Y, Finan JE, Middeldorp JM, Hayward SD. (1997). P32/TAP, a cellular protein that interacts with EBNA-1 of Epstein–Barr virus. *Virology* **236**: 18–29.

Yu L, Loewenstein PM, Zhang Z, Green M. (1995a). *In vitro* interaction of the human immunodeficiency virus type 1 Tat transactivator and the general transcription factor TFIIB with the cellular protein TAP. *J Virol* **69**: 3017–3023.

Yu L, Zhang Z, Loewenstein PM, Desai K, Tang Q, Mao D *et al*. (1995b). Molecular cloning and characterization of a cellular protein that interacts with the human immunodeficiency virus type 1 Tat transactivator and encodes a strong transcriptional activation domain. *J Virol* **69**: 3007–3016.