DNA-Binding and Transactivation Activities Are Essential for TAp63 Protein Degradation

Haoqiang Ying, Donny L. F. Chang, Hongwu Zheng, Frank McKeon and Zhi-Xiong Jim Xiao


Updated information and services can be found at: http://mcb.asm.org/content/25/14/6154

REFERENCES

These include:
This article cites 40 articles, 12 of which can be accessed free at: http://mcb.asm.org/content/25/14/6154#ref-list-1

CONTENT ALERTS
Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: http://journals.asm.org/site/misc/reprints.xhtml
To subscribe to another ASM Journal go to: http://journals.asm.org/site/subscriptions/
DNA-Binding and Transactivation Activities Are Essential for TAp63 Protein Degradation

Haoqiang Ying,1 Donny L. F. Chang, 2 Hongwu Zheng, 1 Frank McKeon,3 and Zhi-Xiong Jim Xiao1,2,*

Department of Biochemistry1 and Department of Medicine,2 Boston University School of Medicine, 715 Albany St.,
Boston, Massachusetts 02118, and Department of Cell Biology, Harvard Medical School,
Boston, Massachusetts 02115

Received 7 December 2004/Returned for modification 13 January 2005/Accepted 26 April 2005

The p53-related p63 gene encodes six isoforms with differing N and C termini. TAp63 isoforms possess a transactivation domain at the N terminus and are able to transactivate a set of genes, including some targets downstream of p53. Accumulating evidence indicates that TAp63 plays an important role in regulation of cell proliferation, differentiation, and apoptosis, whereas transactivation-inert ΔNp63 functions to inhibit p63 and other p53 family members. Mutations in the p63 gene that abolish p63 DNA-binding and transactivation activities cause human diseases, including ectrodactyly ectodermal dysplasia and facial clefting (EEC) syndrome. In this study, we show that mutant p63 proteins with a single amino acid substitution found in EEC syndrome are DNA binding deficient, transactivation inert, and highly stable. We demonstrate that TAp63 protein expression is tightly controlled by its specific DNA-binding and transactivation activities and that p63 is degraded in a proteasome-dependent, MDM2-independent pathway. In addition, the N-terminal transactivation domain of p63 is indispensable for its protein degradation. Furthermore, the wild-type TAp63γ can act in trans to promote degradation of mutant TAp63γ defective in DNA binding, and the TA domain deletion mutant of TAp63γ inhibits transactivation activity and stabilizes the wild-type TAp63 protein. Taken together, these data suggest a feedback loop for p63 regulation, analogous to the p53-MDM2 feedback loop.

The p63 gene is a recently discovered member of the p53 gene family (38). Unlike p53, p63 has six different isoforms. The transactivation (TA) isoforms, which resemble p53, are generated by the use of an upstream promoter and consist of an acidic N-terminal transactivation domain, a central DNA-binding domain, and a C-terminal oligomerization domain (25, 39). The primary amino acid sequences in the DNA-binding domains of p63 and p53 share over 60% identity, whereas in the TA domain they share 25% identity. TAp63 isoforms are capable of transactivating a set of target genes, some of which overlap with targets downstream of p53, including Bax, MDM2, and p21 (39). The ΔN isoforms, produced from an intronic promoter, contain the same DNA-binding and oligomerization domains as the TA isoforms but lack the transactivation domain. The ΔN isoforms also contain a region of 26 amino acids (aa) at the very N-terminal end of the protein (TA2) in which an activation function was recently identified (7). In addition, the ΔN isoforms are capable of forming protein complexes with p53 family proteins to inhibit the function of p53 family members (39). Furthermore, both the TAp63 and ΔNp63 isoforms can undergo alternative splicing to yield three different C-terminal tails (TAp63α, -β, and -γ isoforms and ΔNp63α, -β, and -γ isoforms). Among these isoforms, TAp63γ is the most transactivation-active isoform of p63 (39). In the C-terminal extension of the α-isosforms, there is a sterile alpha motif implicated in protein-protein interactions and thought to be important for mammalian development (32).

Despite their structural homology, the p53 family members have distinctive biological functions. While p53 is a key gatekeeper for genomic stability by regulating cell cycle, DNA damage repair, and apoptosis, p73 and p63 are critical during development and differentiation. In particular, p63 appears to be essential in epithelial and limb development as demonstrated by the mouse models (33). Several dominant human syndromes involving limb development and ectodermal dysplasia have been mapped to the p63 gene, including ectrodactyly, ectodermal dysplasia and cleft lip/palate (EEC) syndrome; nonsyndromic split hand/foot malformation (SHFM); ankyloblepharon, ectodermal dysplasia, clefting (AEC) syndrome;acro-dermato-ungual-lacrimal-tooth (ADULT) syndrome; and limb-mammary syndrome (LMS) (2, 4). Most mutations in the p63 gene identified in EEC patients so far result in amino acid substitutions that are predicted to abolish the DNA-binding capacity of p63. In contrast to p53, the p63 gene is rarely mutated in cancer (12, 21, 25). However, overproduction of ΔNp63 isoforms has been reported in squamous cell carcinoma (10) and in many other types of epithelial tumors (6, 24, 26, 27).

The p53 protein is usually labile in normal cells but is dramatically stabilized upon a variety of cellular stresses. The key negative regulator of p53 is the MDM2 protein, which functions as an E3 ubiquitin ligase for p53 to promote its protein degradation (9, 15). In addition, MDM2 physically binds to the p53 N-terminal transactivation domain, thereby directly inhibiting p53 transactivation activity (5). Importantly, MDM2 is a bona fide target gene downstream of p53. Thus, activation of
p53 up-regulates its own inhibitor (MDM2). This feedback loop ensures that p53 protein levels are maintained at low levels in normal cells.

Here we show that, like p53, the protein stability of the transactivation form of p63, TAp63\(^\gamma\), is also tightly regulated by its DNA-binding and transactivation activities. The N-terminal TA domain is required for its protein degradation independent of MDM2. Our data suggest that TAp63\(^\gamma\) is regulated through a feedback mechanism similar to the p53-MDM2 feedback loop.

**MATERIALS AND METHODS**

**Cell culture and drug treatment.** U2-OS, HeLa, and Saos-2 cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin G/streptomycin sulfate at 37°C in a humidified 5% CO\(_2\) incubator. Wild-type or p53\(^{-/-}\) MDM2\(^{-/-}\) mouse embryonic fibroblasts (MEF) were maintained in DMEM supplemented with 15% FBS. U2-OS-Tet-TAp63\(^\gamma\) and U2-OS-Tet-Luciferase cells were maintained in DMEM supplemented with 10% FBS, 2 \(\mu\)g/ml doxycycline (BD Biosciences–Pharmingen), and 200 \(\mu\)g/ml hygromycin (BD Biosciences–Pharmingen).

**Plasmid construction and transfection.** pcDNA-myc-TAp63\(^\gamma\) and pcDNA-myc-Np63\(^\gamma\) were described previously (39). A BamHI/XhoI or BamHI/XbaI PCR fragment containing TAp63\(^\gamma\) was subcloned into pcDNA-HA or pTRE-tight (BD Biosciences–Pharmingen) to generate pcDNA-HA-TAp63\(^\gamma\) or pTRE-tight-TAp63\(^\gamma\). The pcDNA-myc-TAp63\(^\gamma\) and pcDNA-HA-TAp63\(^\gamma\) plasmids were used to generate deletion mutants or point mutants by site-directed mutagenesis according to the manufacturer’s instructions (Stratagene). The TA domain swapping mutant of TAp63\(^\gamma\), HA-p53TA-p63\(^\gamma\), was generated from pcDNA-HA-TAp63\(^\gamma\) and pcDNA-HA-p53 (40) using overlapping extension as described previously (11). All constructs were confirmed by DNA sequencing.

U2-OS and HeLa cells were transfected with FuGENE 6 (Roche). Saos-2 cells were transfected with CalPhos transfection kit (BD Biosciences–Pharmingen). MEF were transfected with Lipofectamine 2000 (Invitrogen). To examine
protein stability, U2-OS or HeLa cells were transfected with 500 ng of pcDNA-TAp63/H9253 or cotransfected with 500 ng of pcDNA-TAp63/H9253 and 1.0 μg of either pcDNA-TAp63/R304W, pcDNA-ΔTAp63γ, or pcDNA-TAp63γ(FWL-A). Sixteen hours posttransfection, cells were treated with cycloheximide (Sigma) at a final concentration of 50 μg/ml. Cells were collected at time intervals. For MG132 treatment, cells transfected for 30 h were treated with MG132 (20 μM) for 5 h. To generate U2-OS-Tet-TAp63γ and U2-OS-Tet-Luc cells, U2-OS-Tet-off cells (gift from Qiang Yu, Boston University School of Medicine) were cotransfected with 5 μg of pTRE-tight-TAp63γ or pTRE-tight-Luciferase (BD Biosciences–Pharmingen) and 0.5 μg of pTK-Hyg. Twelve hours posttransfection, cells were selected with doxycycline (2 μg/ml) and hygromycin (400 μg/ml) for 4 weeks. Single colonies were selected. All clonal cell lines were tested for induction of TAp63/H9253 or luciferase in the absence of doxycycline by Western blot analysis or luciferase activity assay. A stable U2-OS-Tet-TAp63γ cell line (clone 2) was chosen for further analysis for its effective repression and induction by doxycycline administration or withdrawal.

Luciferase reporter assay and Western blot analysis. For luciferase reporter assay, Saos-2 cells grown in six-well tissue culture dishes at 80% confluence were transfected with 100 ng of either pcDNA-TAp63γ or pcDNA-ΔTAp63γ in the presence of Bax-Luciferase reporter and β-galactosidase plasmids. The luciferase activity was normalized to β-galactosidase activity and reported as an increase in activation (mean ± standard deviation [error bar]). (B) Protein expression was examined by Western blot analysis. V, vector. (C) U2-OS cells transfected with TAp63γ or ΔTAp63γ were treated with cycloheximide (CHX) for the indicated times (in hours). Cell lysates were subjected to Western blot analysis.
Na3VO4. Equal amounts of total protein were subjected to sodium dodecyl

buffer (10 mM HEPES, pH 7.6, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM

dithiothreitol [DTT], 0.5 mM PMSF, 0.2% Nonidet P-40), followed by centrif-

ation at 4,000 rpm for 10 min at 4°C. The pellets were then lysed in hypertonic

buffer (20 mM HEPES, pH 7.6, 1.5 mM MgCl2, 420 mM NaCl, 0.5 mM DTT, 0.5

mM PMSF, 0.5 mM dithiothreitol [DTT], 5% glycerol), followed by centrifugation at 14,000 rpm for 20 min at 4°C. The reaction mixture (total volume of 25 μl) containing 20 μg nuclear

electrophoresis on 4% polyacrylamide gels in Tris-glycine buffer and revealed by autoradiography. Thirty nanograms of oligonucleotide containing the p53-binding consensus site (sc-2579; Santa Cruz Biotechnology) was end labeled in the presence of 25 μCi of [γ-32P]ATP (NEGE002H; New England Nuclear) and 10 U of T4 polynucleotide kinase (M0201; New England BioLabs) for 30 min at 37°C.

**Immunofluorescence staining.** U2-OS cells in six-well plates were cotrans-

fected with either 500 ng of pcDNA vector or pcDNA-TAp63x, or 200 ng of pcDNA-TAp63(R304W), pcDNA-TAp63(C306R), pcDNA-TAp63(FWL-A), or pcDNA-TAp63(ΔTA) in the presence of 50 ng of pEG-GFP using FuGENE 6. Twelve hours posttransfection, cells were trypsinized and 5 × 106 cells were plated onto Lab-Tek II chamber slides (154461; Nalge Nunc International). Twenty-four hours later, cells were fixed in 4% paraformaldehyde (in phosphate-buffered saline, pH 7.4), permeabilized with 0.2% Triton X-100, blocked in phosphate-buffered saline containing 1% bovine serum albumin, and then immuno- 

stained with p63 antibody (sc-8431; Santa Cruz Biotechnology) followed by a Cy3-conjugated goat anti-mouse immunoglobulin G antibody (115-165-146; Jackson ImmunoResearch Laboratory). The cells were counterstained with 4′,6′- 

diamidino-2-phenylindole (DAPI) (300 nM) prior to mounting using ProLong 

Gold Antifade (P36930; Molecular Probes). Staining was visualized using the 

following excitation/emission wavelengths: 358/461 nm for DAPI, 595/615 nm for 

Cy3, and 494/518 nm for green fluorescent protein (GFP). Fluorescent images were captured on a Zeiss Axiovert 200M microscope with Axiosvion v 4.3 program.

**RESULTS**

p63 protein instability correlates with its transactivation activity. It is well documented that the predominant species of p63 in epithelial cells and cancer cells are ΔN isoforms, which lack the transactivation domain at the N terminus, whereas the levels of TA isoforms are very low under physiological condi-

tions (10, 20, 38). We asked whether this phenomenon is rem-

iniscent of p53, whose mutants defective in transactivation activity unavoidably become stabilized. Thus, we examined the protein expression levels of transactivation-potent TAp63x and transactivation-inert ΔNp63x in U2-OS or HeLa cells by Western blot analysis. Antibodies used were specific for p63 (4A4; Santa Cruz), hemagglutinin (HA) (Y-11; Santa 

Cruz), p53 (DO-1; Santa Cruz), Myc (9E10; Santa Cruz), and actin (C-11; Santa 

Cruz).

Electrophoresis mobility shift assay (EMSA). H1299 cells were transfected with 3 μg of p53 or a TAp63x construct. Twenty-four hours posttransfection, nuclear extracts were prepared. Briefly, cells were collected and lysed in hypo-

tonic buffer (10 mM HEPES, pH 7.6, 1.5 mM MgCl2, 10 mM KCl, 0.5 mM

dithiothreitol [DTT], 0.5 mM PMSF, 0.2% Nonidet P-40), followed by centrif-

ugation at 4,000 rpm for 10 min at 4°C. The pellets were then lysed in hypertonic buffer (20 mM HEPES, pH 7.6, 1.5 mM MgCl2, 420 mM NaCl, 0.5 mM DTT, 0.5

mM PMSF, 25% glycerol), followed by centrifugation at 14,000 rpm for 20 min at 4°C. The reaction mixture (total volume of 25 μl) containing 20 μg nuclear

electrophoresis on 4% polyacrylamide gels in Tris-glycine buffer and revealed by autoradiography. Thirty nanograms of oligonucleotide containing the p53-binding consensus site (sc-2579; Santa Cruz Biotechnology) was end labeled in the presence of 25 μCi of [γ-32P]ATP (NEGE002H; New England Nuclear) and 10 U of T4 polynucleotide kinase (M0201; New England BioLabs) for 30 min at 37°C.
frequently located in the central DNA-binding domain (4, 34, 36). Strikingly, these missense mutations correspond very well with the somatic mutational hot spots in the p53 gene, which inactivate p53 DNA binding and p53 growth suppression function. We generated three point mutations in the DNA-binding domains of TAp63γ/H9253γ, TAp63γ/H9253γ (R204W), TAp63γ/H9253γ (R304W), and TAp63γ/H9253γ (C306R). These mutations are found in EEC syndrome and are predicted to abolish the DNA-binding activity.

FIG. 6. The TA domain of p63 is essential for its protein instability independent of MDM2. (A) Saos-2 cells were transfected with wild-type TAp63γ or a chimeric construct (HA-p53TAp63γ) in the presence of Bax-Luciferase and β-galactosidase constructs. The luciferase activity was normalized to β-galactosidase activity and reported as an increase in activation (mean ± standard deviation [error bar]). (B) Saos-2 (p53−/−) or U2-OS (p53−/−) cells transfected with wild-type HA-TAp63γ or HA-p53TA-p63γ were treated by cycloheximide (CHX) for the indicated times (in hours). Cell lysates were subjected to Western blot analysis using antibodies specific for HA tag or actin. (C) Cell lysates (50 μg of total protein) from U2-OS or Saos-2 cells were subjected to Western blot analysis for MDM2 or actin. (D) Wild-type (WT) or p53−/− MEF were transfected with TAp63γ, followed by cycloheximide (CHX) treatment for the indicated times (in hours). Cell lysates were subjected to Western blot analysis using antibody specific for p63.
pressed at much higher levels than wild-type TAp63 DNA-binding activity (see Fig. 5A) and, as expected, no degradation of p63 (4). Indeed, all three mutant proteins exhibited no specific DNA-binding activity of TAp63 (4). Thus, these data indicate that the transcriptional activity of TAp63γ is critical for its protein instability.

The FWL motif in the TA domain is essential for p63 protein degradation. The p53-MDM2 feedback regulation is a well-established mechanism for the control of p53 protein levels. Three hydrophobic amino acids within the p53 N terminus, F19 W23 L26, which are buried in a cleft within the interface between p53 and MDM2, are critical for the MDM2-mediated degradation of p53 protein (16). Despite a poor homology (25%) among the p53 family members in the N-terminal transactivation domain, the FWL motif is well conserved (Fig. 4A). Therefore, we examined whether the FWL motif is also critical for p63 degradation. As shown in Fig. 4, the triple point mutant, TAp63γ(FWL-A) in which these three amino acid residues (F16, W20, and L23) are replaced by alanine, retained strong DNA-binding activity (Fig. 5A) and partial transactivation activity in a dose-dependent manner (Fig. 4B and 4C), yet the protein was highly stable (Fig. 4C). These data suggest either that the partial transactivation activity is insufficient to promote p63 protein degradation or that the FWL motif is critical for p63 protein turnover. To address this question, we generated a HA-tagged chimeric protein HA-p53TA-p63γ in which the TA domain (aa 1 to 64) of TAp63γ is replaced with the TA domain (aa 1 to 45) of p53. As shown in Fig. 6, p53TA-p63γ was fully competent in transactivation (Fig. 6A). Strikingly, it was stable in Saos-2 cells (Fig. 6B), indicating that the transactivation activity alone is not sufficient for protein degradation. However, in contrast to remarkable protein stability in Saos-2 cells, p53TA-p63γ protein was rapidly turned over in U2-OS cells (Fig. 6B). Since U2-OS cells contain wild-type p53 and express much higher levels of MDM2 in comparison to the p53 null Saos-2 cells (Fig. 6C), it is likely that degradation of the p53TA-p63γ chimeric protein is dependent on MDM2, which binds to the TA domain of p53. Of note, the FWL motif is important for p53 interaction with MDM2 (16). Thus, the conserved FWL motif in the TA domain of TAp63γ may serve as a binding site for MDM2, which might in turn promote p63 degradation. However, the ectopically expressed TAp63γ exhibited similar protein half-lives in wild-type MEF and p53−/− MDM2−/− MEF (Fig. 6D), indicating that degradation of TAp63γ does not require MDM2. Thus, the FWL motif in the TA domain of TAp63γ is critical for its protein degradation in an MDM2-independent manner.

Wild-type TAp63γ can act in trans to promote degradation of the p63 DNA-binding mutant, but not the p63 TA domain mutants. Since both the transcriptional activity and the FWL motif of TAp63γ are crucial for its protein instability, it is very possible that there may be a feedback control mechanism for regulation of p63 protein levels, analogous to the p53-MDM2 feedback loop. This hypothesis would predict that TAp63γ transactivates expression of an unidentified target gene whose product interacts with the FWL motif in the TA domain to promote TAp63γ protein degradation via a proteasome-dependent pathway. Accordingly, the wild-type TAp63γ should be able to act in trans to promote the degradation of mutant...
p63 protein defective in DNA binding, but not the mutant p63 lacking the TA domain or the mutant p63 with a defective FWL motif. Indeed, in cotransfection experiments, the protein half-life of HA-tagged TAp63\textsubscript{HA9253}(R304W) was decreased by 50% in the presence of Myc-tagged wild-type TAp63\textsubscript{HA9253}, while the half-life of HA-tagged TAp63\textsubscript{HA9253} or TAp63\textsubscript{HA9253}(FWL-A) was not affected by the presence of wild-type TAp63\textsubscript{HA9253} (Fig. 7). A similar phenomenon was observed using an inducible U2-OS-Tet-TAp63\textsubscript{HA9253} stable cell line. Upon withdrawal of doxycycline, expression of TAp63\textsubscript{HA9253} was markedly induced (Fig. 8C). Induction of TAp63\textsubscript{HA9253}, but not induction of luciferase in the control cell line, led to a significant decrease in the protein half-life (\textasciitilde2 h) of HA-TAp63\textsubscript{HA9253}(R304W) (Fig. 8A and B).

It has been shown that nontransactivation ΔTAp63 isoforms are predominantly expressed in cancer cells and in epithelial progenitor cells (10, 20, 38). We therefore investigated the influence of transactivation-inert p63 mutant ΔTAp63\textsubscript{γ} on the wild-type TAp63\textsubscript{γ} protein stability. As shown in Fig. 9, coexpression of ΔTAp63\textsubscript{γ} led to a marked suppression of transactivation activity and significant stabilization of TAp63\textsubscript{γ} protein. Interestingly, coexpression of DNA-binding-defective TAp63\textsubscript{γ} (R304W) exhibited a much lower inhibitory effect on the TAp63\textsubscript{γ} transactivation activity and no significant effect on the stability of TAp63\textsubscript{γ} protein (Fig. 9). Thus, nontransactivation ΔTAp63\textsubscript{γ} can effectively function in a dominant-negative fashion in suppression of transactivation activity of wild-type TAp63\textsubscript{γ} and modulate the stability of transactivation p63 isoforms.

**FIG. 8.** Induction of TAp63\textsubscript{γ} promotes degradation of p63 DNA-binding mutant. Doxycycline-repressible stable U2-OS cells, U2-OS-Tet-TAp63\textsubscript{γ} and U2-OS-Tet-Luc, were transfected with HA-TAp63\textsubscript{γ}(R304W). Transfected cells were grown in the presence (+) or absence (−) of doxycycline for 24 h, followed by cycloheximide (CHX) treatment for the indicated times (in hours). Cell lysates were subjected to Western blot (WB) analysis using antibodies specific for HA tag or actin (A). The data in panel A were subjected to quantitative analysis and presented as a percentage of remaining protein levels (B). Induction of TAp63\textsubscript{γ} expression in U2-OS-Tet-TAp63\textsubscript{γ} stable cells was assessed by Western blot analysis for p63 (C).

**DISCUSSION**

Although the regulation of p53 protein stability has been extensively studied, how p63 protein stability is regulated is largely unknown (19). Here we demonstrate that the transactivation-potent species of p63, TAp63\textsubscript{γ}, is tightly regulated likely through a feedback mechanism, analogous to the p53-Mdm2 feedback loop (Fig. 10). We show that both DNA-binding and transactivation activities are essential for TAp63\textsubscript{γ} instability and, furthermore, that the unique features of the TAp63\textsubscript{γ} TA domain play an important role in protein degradation. Moreover, we show that the wild-type TAp63\textsubscript{γ} can act in trans to induce the degradation of the p63 DNA-binding mutant and that the p63 deletion mutant lacking the TA domain can stabilize the TAp63\textsubscript{γ} protein through its dominant-negative effect.

p53 is frequently mutated in human cancers with a high frequency of point mutations, referred to as hot spots, in the DNA-binding domain. These mutant p53 proteins are usually stable, since they are incapable of inducing the expression of MDM2 (22). Strikingly, many of the naturally occurring p63 mutations, especially those found in human EEC syndrome, correspond very well with hot spot mutations found in the p53 gene (2, 4). For instance, the missense mutations creating R204W, R304W, and C306R substitutions in the p63 DNA-binding domain correspond to R175, R273, and C275 of p53, respectively. These DNA-binding-defective TAp63\textsubscript{γ} proteins are highly stable, thus supporting the notion that specific
DNA-binding activity, while being a prerequisite for TAp63γ protein degradation. Alternatively, it is possible that interaction of TAp63γ with DNA is required for its protein degradation in a manner similar to some transcription factors, such as estrogen receptor, Myc, and VP16, whose DNA-binding activity is critical for their protein degradation (13, 28, 29, 35). However, our data reveal that wild-type TAp63γ contains a unique transactivation domain that is not required for the degradation of TAp63γ. Interestingly, a recent study reported that the F16-W20-L23 motif functions as a protein-protein interaction site with which the p63 protein can induce the degradation of TAp63γ. Additionally, three hydrophobic amino acids (F19-W23-L26) within the p53 N terminus are critical for the MDM2-mediated degradation of p53 protein (16). Despite the poor homology between the p53 family members, those three amino acids are well conserved in p53, p63, and p73; this may mean that MDM2 might also be able to bind the FWL motif and promote p63 protein degradation. However, our data indicate that MDM2 is not required for p63 degradation, as evidenced by the observation that p63 protein can be degraded equally well in p53−/−/MDM2−/− MEF and wild-type MEF. These data are consistent with the observations indicating that overexpression of MDM2 does not lead to p63 protein instability (3, 18).

The notion that p63 is regulated by a feedback loop is further supported by the observation that the ΔTAp63γ mutant can significantly inhibit the transactivation activity of the wild-
type TAp63α and lead to its protein stabilization. It is possible that excess expression of ΔTAp63α may occupy the promoter regions to which the transactivation p63 normally binds and thereby block TAp63-mediated transcription, which may in turn suppress the expression of proteins involved in p63 protein degradation (Fig. 10). Interestingly, a recent study showed that the transactivation activity of p73 is also critical for p73 protein degradation (37). It is plausible that cells may have developed similar feedback regulation mechanisms during evolution in regulation of p53 family protein expression despite their distinct biological functions.

It has been established that certain unstable transcription factors contain the degron sequences in the transactivation domains so that transcription can be coupled to proteolysis (17, 23, 30). Our data demonstrated that TAp63α degradation is also tightly coupled to its transcriptional activity, which may partially explain the low levels of expression of transactivation-active p63 isoforms in most tissues and cell lines. Recent studies have demonstrated that among the p63 proteins TAp63α isoforms are the first to be expressed during embryogenesis and are required for commitment to an epithelial stratification program. Δp63α is the predominant isoform expressed in the basal cells of many epithelial tissues (14, 20). Since TAp63α isoforms seem to inhibit terminal differentiation, their activities must be counterbalanced by various mechanisms, such as transcription-coupled degradation of TAp63α and expression of Δp63α in neutralizing TAp63α, to allow cells to respond to signals required for the maturation of embryonic epidermis. It is possible that uncontrolled expression of transactivation-potent p63 isoforms leads to adverse effects on cells, since TAp63α may be shown as an inducer for apoptosis (8, 39).

Interestingly, the p63 mutations in the DNA-binding domain observed in several dominant human syndromes are highly stable. It is conceivable that these mutations may regulate the function of p53 family proteins to disrupt normal cell proliferation and development. Although our data indicate the TAp63α protein stability is tightly controlled by its transcriptional activity, the precise molecular mechanism, however, remains to be elucidated.

ACKNOWLEDGMENTS


