

p63 is upstream of IKK α in epidermal development

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Summary

The epidermis, the outer layer of the skin composed of keratinocytes, develops following the action of the transcription factor p63. The mouse *Trp63* gene contains two promoters, driving the production of distinct proteins, one with an N-terminal trans-activation domain (TAp63) and one without (Δ Np63), although their relative contribution to epidermal development is not clearly established. To identify the relative role of p63 isoforms in relation to IKK α , also known to be essential for epithelial development, we performed both molecular and in vivo analyses using genetic complementation in mice. We found that the action of TAp63 is mediated at the molecular level

by direct and indirect transactivation of IKK α and Ets-1, respectively. We also found that Δ Np63 upregulates IKK α indirectly, through GATA-3. Our data are consistent with a role for p63 directly upstream of IKK α in epithelial development.

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Key words: p63, Epidermis, Cornification, Skin, Differentiation, Development

Introduction

The epidermis is a multi-layered, stratified epithelium continuously regenerated by terminally differentiating keratinocytes (Candi et al., 2005; Fuchs and Watt, 2003; Owens and Watt, 2003), in which a major role is played by p63 (Yang et al., 1998), a member of the p53 family (Melino et al., 2002; Melino et al., 2003; Yang et al., 2002; Yang and McKeon, 2000). Indeed, mutations in the human *TP63* gene cause skin and limb defects (Celli et al., 1999), and p63^{-/-} mice have no epidermis, no limbs and die at birth as a result of dehydration (Mills et al., 1999; Yang et al., 1999).

The expression of p63 proteins originates from two promoters, giving rise to two distinct isoforms, TAp63 and Δ Np63. In addition, both isoforms undergo alternative splicing at the C-terminus producing different TAp63 and Δ Np63 isoforms, respectively named α , β and γ , with α being the longest. Although p63 is involved in epithelial development (Mills et al., 1999; Yang et al., 1999), the relative contribution of these different isoforms to epidermal formation has been established at the molecular level only partially (Candi et al., 2006; Koster et al., 2004).

The formation of the epidermis requires the action of the I κ B kinase- α (IKK α). IKK α shows both protein-kinase-dependent and -independent effects; the kinase-independent function is required for epidermal keratinocyte differentiation, skeletal and craniofacial morphogenesis, as shown by the IKK α ^{-/-} mice (Hu et al., 1999; Li et al., 1999; Sil et al., 2004). Thus, loss of IKK α prevents terminal differentiation of keratinocytes by blocking the expression of late differentiation markers, such as loricrin and filaggrin (Hu et al., 1999; Hu et al., 2001; Li et al., 1999), and expression of IKK α , under the basal layer promoter cytokeratin 14 (K14) in IKK α ^{-/-} mice, produced efficient

rescue of the major morphological abnormalities (Sil et al., 2004). IKK α is also required for normal whisker and tooth development, where, in the developing oral cavity, the epithelium invaginates inward into the underlying mesenchyme (Ohazama et al., 2004). This appears to be independent of NF- κ B. IKK α knockout mice also show an abnormal tooth cusp morphology very similar to *EdaA1*, *Edar* and *Edaradd* mutants. Changes in *Notch1*, *Notch2*, *Wnt7b* and *Shh* gene expression in incisor epithelium of IKK α -deficient mice suggest that this IKK α function is downstream of *EdaA1/Edar/Edaradd* and is mediated by *Notch/Wnt/Shh* signaling pathways. Interestingly, p63 is also required for whisker and tooth development (Laurikkala et al., 2006; Rufini et al., 2006). Here, *Bmp7*, *Fgfr2b*, *Jag1*, *Notch1* and *Edar* transcripts are co-expressed with Δ Np63, and are absent in p63^{-/-} mice (Laurikkala et al., 2006). The involvement of p63 and IKK α in the development of the epidermis, whiskers and teeth, raises the question of the reciprocal molecular relationship between p63 and IKK α .

Here, we provide evidence that p63 is directly upstream of IKK α in epidermal development. Using HA-tagged p63-inducible Tet-on Saos-2 cell lines (see Gressner et al., 2005), we demonstrate that TAp63 induces the expression of IKK α , through the p53-like responsive element on its promoter. In addition, TAp63 also drives the expression of Ets-1, another factor which transactivates the IKK α promoter. Finally, we identified four GATA-3 binding sites located in the human IKK α proximal promoter, and because both TAp63 and Δ Np63 are able to transactivate the GATA-3 promoter, this provides a further indirect mechanism allowing TAp63 and Δ Np63 to modulate IKK α expression. Consequently, we observed that the double complemented mice [transgenic TAp63 α and

Δ Np63 α mice under the keratin 5 promoter crossed into p63^{-/-} mice, thus named p63^{-/-} Δ N;TA, generated in our laboratory (Candi et al., 2006)] show higher levels of epidermal IKK α expression compared with p63^{-/-} mice. This provides evidence for p63 being directly upstream of IKK α in epidermal development.

Results and Discussion

IKK α is a transcriptional target of p63

In order to elucidate the molecular mechanism through which p63 regulates the formation of the epidermis, we investigated its ability to induce IKK α . The human IKK α promoter contains three specific p53-like responsive elements that could be recognised by p63 proteins (Fig. 1A). This promoter region was cloned in a plasmid upstream of a luciferase reporter gene. We observed that, in Tet-inducible Saos-2 cells (Candi et al., 2006; Gressner et al., 2005), TAp63 α significantly increased luciferase activity in a dose-dependent manner (Fig. 1B, lanes 2-3), whereas Δ Np63 α did not (Fig. 1B, lanes 4-5). In addition, TAp63 β and TAp63 γ also significantly increased luciferase activity (Fig. 1C, lanes 3-4) compared with Δ Np63 β and Δ Np63 γ isoforms (Fig. 1C, lanes 6-7). Transactivation of the

IKK α promoter by TAp63 was also demonstrated in HEK293 cells (data not shown). Microarray studies, performed on TAp63- and Δ Np63-inducible Saos-2 cells (Candi et al., 2006; Gressner et al., 2005), have shown that TAp63 α (3.2 times the control level by microarray activation; 6.3 times the control level by real-time PCR at 24 hours), but not Δ Np63 α (0.3-fold over control level by microarray activation; 1.1 times the control level by real-time PCR at 24 hours) also drives expression of Ets-1 (Candi et al., 2006) (data not shown). Expression of Ets-1 alone in both types of Saos-2 cells activates the IKK α promoter (Fig. 1B, lanes 1, 6) and the IKK α promoter contains an Ets-1 consensus motif (Fig. 1A). Co-expression of TAp63 α with Ets-1 results in a greater enhancement of IKK α promoter activity compared with that produced by TAp63 α alone (Fig. 1B, lanes 7, 3). However, co-expression of Δ Np63 α with Ets-1 did not increase the activity of the IKK α promoter over that produced by Ets-1 alone (Fig. 1B, compare lanes 8 and 6).

Using the inducible HA-tagged TAp63 α and Δ Np63 α Saos-2 cell lines (Gressner et al., 2005), we demonstrated that TAp63 α induces IKK α through direct binding to the previously described p53-like responsive elements in its promoter (Gu et al., 2004) as demonstrated by chromatin immunoprecipitation

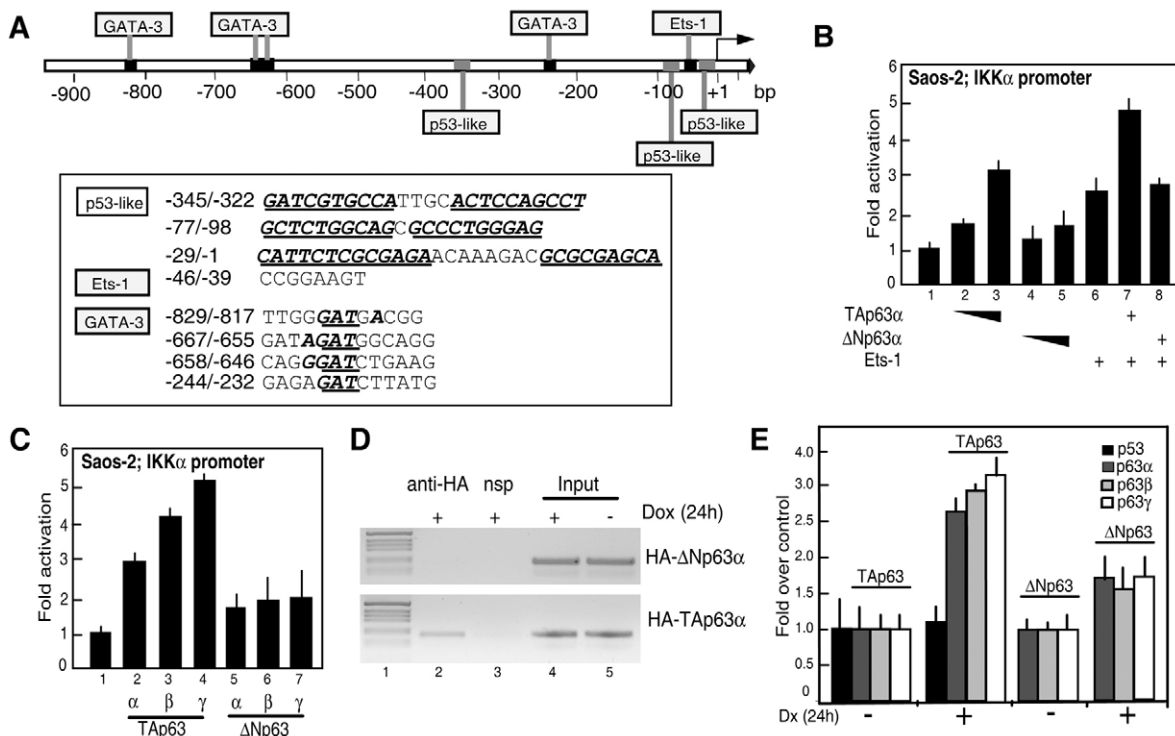


Fig. 1. TAp63 isoforms bind and transactivate the IKK α promoter. (A) Schematic structure of the IKK α promoter showing the different putative p53-like, Ets-1 and GATA-3 responsive elements. (B) Luciferase assay analysis showing the ability of TAp63 α to induce IKK α expression. The ratios of IKK α promoter to transcription factor are: IKK α :TAp63 α / Δ Np63 α , 1:0.5; IKK α :Ets-1, 1:2; IKK α :TAp63 α / Δ Np63 α :Ets-1, 1:2:2. The luciferase assay shown was performed in Saos-2 cells, and similar results were also obtained in HEK293 cells (data not shown). Results are shown as mean \pm s.d. of three independent experiments. (C) Luciferase assay analysis showing the ability of TAp63 and Δ Np63 isoforms to transactivate IKK α in Saos-2 cells. Results are similar to those obtained in Saos-2 Tet-on inducible cells in B. The ratio of IKK α promoter to TAp63/ Δ Np63 isoforms was 1:2. Results are shown as mean \pm s.d. of three independent experiments. (D) ChIP showing the binding of p63 protein to the IKK α promoter. Lane 1, marker; lane 2, specific antibody (anti-HA); lane 3, non-specific antibody (nsp); lane 4, input+Dox; lane 5, input-Dox, (representative result of two independent experiments is shown). (E) Real-time PCR performed on Dox (Tet-on) inducible Saos-2 clones. TAp63 isoforms already induce a significant increase of IKK α RNA upon 24 hours of Dox treatment, whereas Δ Np63 α and p53 do not significantly change IKK α RNA levels. Results are mean \pm s.d. of three independent experiments.

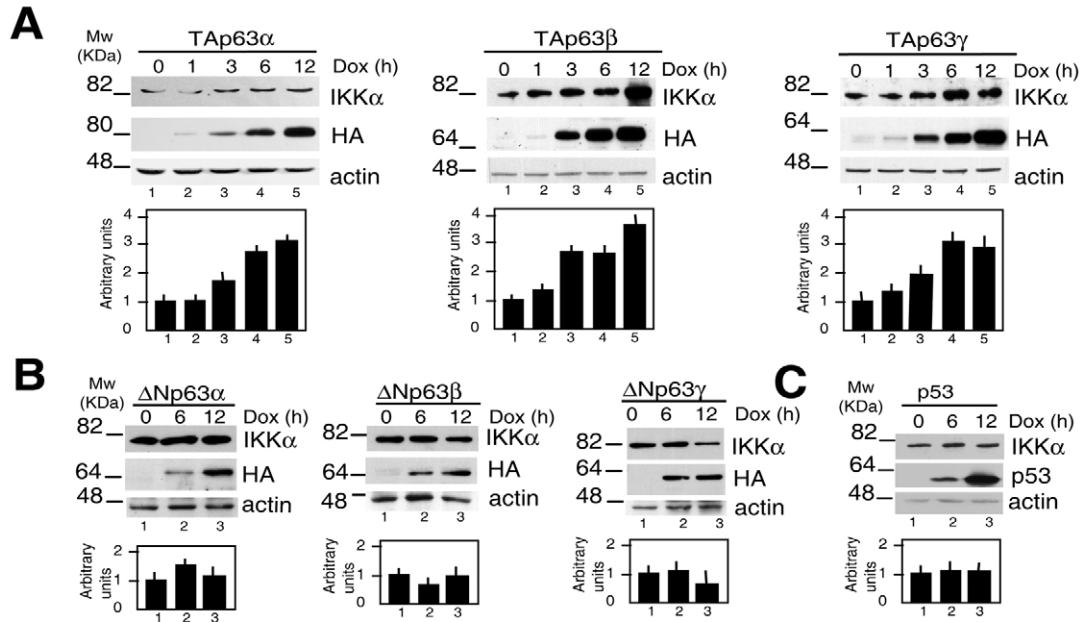


Fig. 2. TAp63 isoforms induce IKK α protein expression. (A) Western blot in Saos-2 Tet-on inducible cell lines (Dox 2 μ g/ml) overexpressing TAp63 or Δ Np63 isoforms. All TAp63 isoforms show an induction of IKK α at the protein level (from 1.3- to 3.7-fold). The actin western blot is shown in the lower panel as a loading control. IKK α expression was normalised to actin by densitometry and results are reported as fold change over zero time (see graphics below actin bands). (B) Δ Np63 isoforms expression do not change (Δ Np63 α) or produce a minor downregulation (Δ Np63 β and ψ) of IKK α protein after 12 hours of Dox induction. IKK α expression was normalised to actin by densitometry and results are reported as fold change over zero time. (C) Western blot in Saos-2 Tet-on inducible cell lines (Dox 2 μ g/ml) overexpressing p53. p53 does not induce IKK α at either protein or RNA level (see also Fig. 1) after 6 hours and 12 hours of induction. IKK α expression was normalised to actin by densitometry and results are reported as fold change over the level at zero time. All results obtained from the densitometry are shown as mean \pm s.d. from three independent experiments.

(ChIP) experiments (Fig. 1D). Immunoprecipitation, using anti-HA antibodies, of protein-DNA complexes containing HA-tagged TAp63 but not Δ Np63, yielded DNA fragments containing IKK α sequences (Fig. 1D, compare lanes 2 in lower and upper panels). No IKK α sequence was amplified from immunoprecipitates prepared with the control anti-K5 antibody (Fig. 1D, compare lanes 2 and 3). RT-PCR analysis of IKK α expression levels in the inducible TAp63 and Δ Np63 Saos-2 cell lines confirms that expression of all TAp63 isoforms

upregulates the transcription of IKK α mRNA after 24 hours of induction with Dox, whereas Δ Np63 isoforms and p53 do not (Fig. 1E).

The induction of IKK α by TAp63 isoforms was also confirmed by western blot in different Saos-2-inducible clones (Fig. 2A,B). Induction of HA-tagged TAp63 α , β and ψ in inducible Saos-2 cells is associated with a parallel induction of IKK α at the protein level, which is detectable after 3 hours of Dox treatment. No change in IKK α expression was elicited by p53 (Fig. 2C). IKK α promoter activation by TAp63 but not Δ Np63 isoforms was also confirmed by transient transfection experiments in human keratinocytes, HaCaT

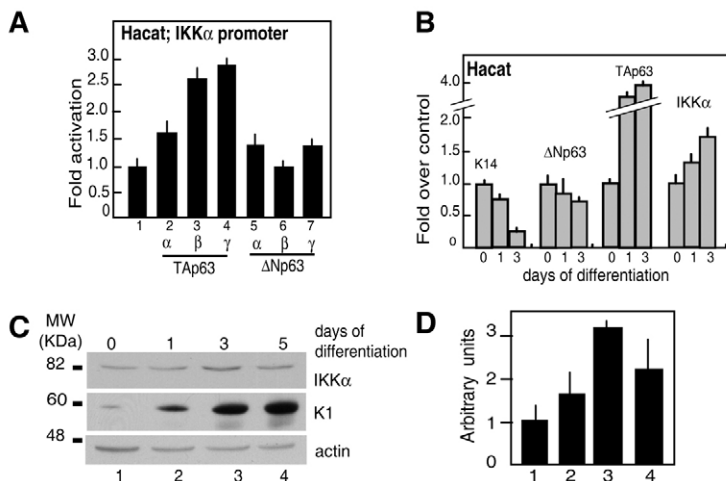


Fig. 3. Regulation of IKK α during keratinocyte differentiation. (A) Luciferase assay showing the ability of p63 isoforms to transactivate IKK α in HaCaT cells. The ratio IKK α promoter to TAp63/ Δ Np63 isoforms was 1:2. Results are shown as mean \pm s.d. of three independent experiments. (B) Quantitative real-time PCR performed on HaCaT cells induced to differentiate in high Ca $^{2+}$ for 1 and 3 days. Δ Np63 α and K14 mRNA decreases indicating that the cells underwent differentiation. Results are shown as mean \pm s.d. from three independent experiments. (C) Western blot using anti-IKK α and anti-keratin 1-antibody (K1) on HaCaT cells induced to differentiate in high Ca $^{2+}$ for 1 to 5 days. K1 is shown as control for keratinocyte differentiation; actin is shown as loading control. (D) Quantification of the western blot by densitometry.

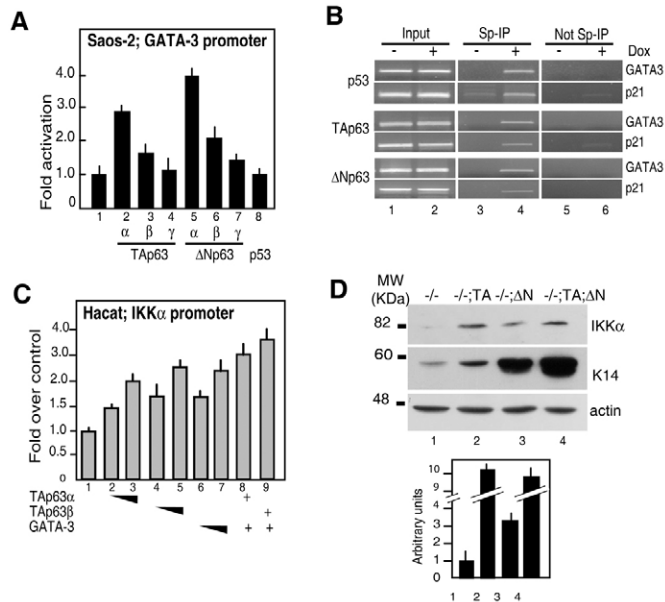


Fig. 4. IKK α regulation by GATA-3, and overexpression in Δ Np63/TAp63 genetically complemented mice. (A) Galactosidase assay showing the ability of TAp63 α and Δ Np63 α to activate the GATA-3 promoter. The ratio of GATA-3 promoter to transcription factor is 1:3. The in vitro transcription assay shown was performed in Saos-2 cells, and similar results were also obtained in HEK293 cells (data not shown). Results are shown as mean \pm s.d. from three independent experiments. (B) ChIP showing the binding of p53 and p63 proteins to the GATA-3 promoter. Lanes 1 and 2, input; lanes 3 and 4, specific antibody (Sp-IP, anti-GATA-3 and anti p21); lanes 5 and 6, non-specific antibody (Not Sp-IP). A representative result of two independent experiments is shown. (C) Luciferase assay showing the ability of GATA-3 to directly induce the IKK α promoter. The ratio of IKK α promoter to transcription factor is 1:1 and 1:3. In lanes 8 and 9 the ratio was 1:1:1. The in vitro transcription assay shown was performed in HaCaT cells, and similar results were also obtained in HEK293 cells (data not shown). Results are shown as mean \pm s.d. from three independent experiments. (D) Western blot of epidermal cell extracts from p63^{-/-}, p63^{-/-};TA, p63^{-/-};ΔN, p63^{-/-};ΔN;TA mice (see 13). Double p63^{-/-};ΔN;TA complemented mice, as well as p63^{-/-};TA mice, show higher levels of IKK α expression in vivo compared with p63^{-/-} mice. As loading control we detected actin. Lower panel shows the normalisation of the induction of IKK α to actin protein level.

cells (Fig. 3A, lanes 1, 2-4 for TAp63; lanes 1, 5-7 for Δ Np63) and NHEK (data not shown).

By real-time PCR analysis (Fig. 3B) we confirmed that TAp63 and IKK α mRNA increase during keratinocyte differentiation, whereas, as expected during epidermal differentiation (26), Δ Np63 and K14 mRNA levels decrease. The increase of IKK α mRNA was also confirmed by western blot (Fig. 3C) in differentiating keratinocytes. Here, protein levels increase ~threefold after 3 days of treatment with Ca²⁺ (Fig. 3D).

Indirect regulation of IKK α by p63 via GATA-3

To determine additional potential indirect mechanisms of p63-mediated regulation of IKK α , we analysed the human IKK α

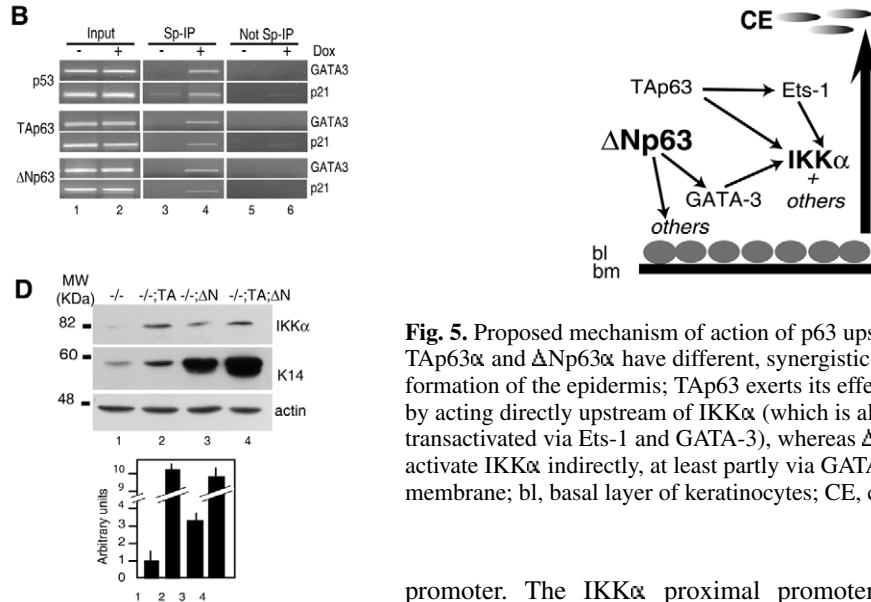


Fig. 5. Proposed mechanism of action of p63 upstream of IKK α . TAp63 α and Δ Np63 α have different, synergistic functions in the formation of the epidermis; TAp63 exerts its effects, at least in part, by acting directly upstream of IKK α (which is also indirectly transactivated via Ets-1 and GATA-3), whereas Δ Np63 can only activate IKK α indirectly, at least partly via GATA-3. bm, basal membrane; bl, basal layer of keratinocytes; CE, cornified envelope.

promoter. The IKK α proximal promoter contains several binding sites for transcription factors which play important roles during skin development and differentiation (see supplementary material Table S1 and Fig. S1). Beside Ets-1, we decide to investigate the possible role of GATA-3, an important mediator of skin and hair development (Kaufman et al., 2003), in regulating IKK α expression, based on the observation that GATA-3 contains a p53-like consensus binding site in its proximal promoter region (-242 to -262 bp). Unlike the IKK α promoter, both TAp63 α and Δ Np63 α significantly increased luciferase activity of the GATA-3 promoter (Fig. 4A, lanes 2, 5). Interestingly, p63 β and p63 γ isoforms are less efficient in transactivating this promoter. Using the inducible HA-tagged TAp63 α and Δ Np63 α Saos-2 cell lines, we demonstrated that both p63 isoforms (p53 included as positive control), directly bind to the putative p53-like responsive element in GATA-3 promoter as demonstrated by the ChIP experiments (Fig. 4B). Immunoprecipitation, using anti-HA antibodies, of protein-DNA complexes containing HA-tagged TAp63 and Δ Np63, yielded DNA fragments containing GATA-3 sequences. No GATA-3 sequence was amplified from immunoprecipitates prepared with the control anti-K5 antibody (compare Not Sp-IP with Sp-IP). As a positive control we also performed ChIP experiments using p21 (Fig. 4B).

Finally, using a luciferase assay using the IKK α promoter, we observed that GATA-3 transactivates IKK α in a dose-dependent manner (Fig. 4C, lanes 1, 6-7). Moreover, promoter activity increases when GATA-3 is co-transfected with p63 (Fig. 4C, lanes 8-9).

IKK α mediates the function of p63 in genetically complemented mice

To elucidate the individual role of p63 isoforms in the development of the epidermis, we generated transgenic mice expressing either TAp63 α and/or Δ Np63 α under the control of a keratinocyte specific promoter (keratin 5); we then crossed these mice into a p63^{-/-} background, generating single (p63^{-/-};TA and p63^{-/-};ΔN) and double complemented (p63^{-/-};ΔN;TA) mice (Candi et al., 2006). Supplementary material Fig. S2 shows the histology of the epidermis both in

the wild type, knockout and genetically complemented mice. The p63^{-/-};ΔN as well as the p63^{-/-};ΔN;TA complemented mice showed greater, though still not normal, formation of the epidermis (Candi et al., 2006). We therefore took advantage of these mice to evaluate the in vivo relationship between p63 and IKKα.

In the single complemented mice, selective reintroduction of both TAp63α and ΔNp63α was associated with increased IKKα expression compared with p63^{-/-} mice, although the increase was greater with TAp63 complementation (tenfold with TAp63 versus 3.5-fold with ΔNp63; Fig. 4D, lanes 2 and 3). As expected, IKKα expression was also enhanced in the double complemented mice (Fig. 4D, lane 4).

The function of p63 in epithelial development is mediated by IKKα

TP63 is expressed very early in keratinocyte differentiation (Mills et al., 1999; Yang et al., 1999). The ΔNp63 protein is expressed in the basal layer of the human epidermis (Laurikkala et al., 2006; Nylander et al., 2002) and, in both zebra fish and mice, determines the outgrowth of the epidermis (Laurikkala et al., 2006; Lee and Kimelman, 2002). Recently, a distinct contribution of the TAp63 protein has also been demonstrated in mice (Candi et al., 2006). As yet, however, relatively few downstream targets of p63 are known (Candi et al., 2006; Ihrie et al., 2005; Laurikkala et al., 2006) although one of these is fos, a member of the AP1 complex crucial for skin differentiation (Wu et al., 2003) and another is PERP, crucial for epithelial stratification, being localised in desmosomes (Ihrie et al., 2005). Here, we demonstrate that TAp63 and ΔNp63 regulate expression of the gene encoding IKKα, which is also crucial for formation of the epidermis. This regulation is both direct, through one of the three p53 consensus motifs in the IKKα promoter, and indirect, (1) through TAp63 induction of Ets-1, and (2) through TAp63 and ΔNp63 induction of GATA-3, which subsequently drives IKKα expression (Fig. 5). However, as suggested by recent studies (Ihrie et al., 2005; Koster et al., 2004), and by the limited reversion of the p63-null epidermal phenotype by selective TAp63 and ΔNp63 complementation (Candi et al., 2006), other genes must contribute to the proliferative and differentiation potential of the epidermis.

Conclusion

Within the limits of our experimental model, our overall conclusion is that the p63/IKKα pathway is essential for epidermal development. These data are important in understanding the molecular mechanisms of development and maintenance of epithelia and thus have important clinical implications (McKeon, 2004).

Materials and Methods

Cell culture

Primary keratinocytes were isolated as described (Yuspa et al., 1989) from the skin of E19.5 transgenic neonates. Saos-2 cells with doxycycline (Dox)-inducible expression of HA-TAp63a, -b, -g and HA-DNp63a, -b, -g were generated as described previously (Gressner et al., 2005).

Mice

The recombination vector contained the K5 promoter (from Manfred Blessing, Joannes Gutemberg University, Mainz, Germany), the PolyA⁺ signal and N-tagged (hemagglutinin antigen, HA) mouse TAp63a or DNp63a (Breuhahn et al., 2000). Transgenic mice on a C57/B6 background were backcrossed with p63^{+/-} mice (Yang

et al., 1999) to generate genetically complemented mice (Candi et al., 2006). Histology was performed according to standard procedures.

Western blots

HaCaT cells were differentiated by addition of Ca²⁺ and collected after 1 and 3 days of treatment. Saos-2-inducible cells were cultured and treated as described previously (Gressner et al., 2005). Cells were lysed as previously reported (Candi et al., 2006) and after extraction, 50 mg protein were separated by SDS-PAGE and transferred onto PVDF membranes; blots were performed using standard procedures. Primary antibody dilutions were: monoclonal anti-p63 (1:200), polyclonal anti-HA (Y-11, Santa Cruz, 1:100), polyclonal anti-IKKα (1:200), polyclonal anti-K14 (1:300). Normalisation was achieved with a polyclonal anti-tubulin (H-235, Santa Cruz, 1:1000 dilution) or with a goat anti-actin antibody (C-11, Santa Cruz, 1:1000 dilution). Proteins were detected using the ECL method.

Real-time PCR assay

RNA was extracted from control and Dox-induced SaOs-2 Tet-on HA-tagged TAp63 and -DNp63 clones, using Trizol (Invitrogen, Carlsbad, CA). Real-time PCR was performed as previously described (Candi et al., 2006). Primer sequences are available upon request. Relative quantification of gene expression was performed using ABI 7500 SDS software v 1.31.

ChIP and transcription assay

This was performed according to a previous published protocol (Munarriz et al., 2004). Samples (1.5 × 10⁶ cells) were incubated with 2 mg monoclonal anti-HA (16B12, Covance) or monoclonal anti-p63 (Ab4, Neomarkers). The negative control was incubated with 2 mg mouse anti-K5 (Santa Cruz). DNA samples were then analysed with 38 cycles of PCR to amplify IKKα promoter sequences (94°C for 30 seconds, 58°C for 40 seconds, 72°C for 40 seconds). For IKKα amplification we used specific forward and reverse primers (5'-GTGGTTCCGTTACGCCCT-3' and 5'-TGCTCGCGCTCTTTG-3'). The resulting product was 188 bp and contained the last two putative p53 consensus sequences as well as the Ets-1 binding motif in the IKKα promoter. The following specific oligos were used to determine p53/p63 binding sites of p21 and GATA-3 genes: p21 site F(5'-ATGTATAGGAGCGAAG-GTGCA-3'); p21 site R(5'-CCTCCTTTCTGTGCCTGAAACA-3'); GATA-3 site F(5'-GAATCCCTCCTGCCTGTCC-3'); GATA-3 site R(5'-CTTACCTCCAC-CCCATCC-3'). For luciferase assay in Saos and HaCaT cells, we used the reporter plasmid containing the luciferase gene under the control of IKKα promoter and the expression vectors encoding for TAp63α, β, γ and ΔNp63α, β, γ (see legend figures for ratios). When needed, the pcDNA empty vector (Invitrogen) was added to reach the total amount of DNA (400 ng) used in each transfection. In all cases, 10 ng of *Renilla* luciferase vector (pRL-CMV; Promega, Madison, WI) were co-transfected, as a control of transfection efficiency. Luciferase activities of cellular extracts were measured, by using a Dual Luciferase Reporter Assay System (Promega); light emission was measured over 10 seconds using an OPTOCOMP 1 luminometer. Efficiency of transfection was normalised using *Renilla* luciferase activity. For β-galactosidase assay, cells were transiently transfected with a reporter construct containing GATA-3 promoter (-308pLacZ, kindly provided by J. D. Engel, University of Michigan Medical School, MI) and expression vectors encoding p53, p63 isoforms and pcDNA3.1 (see legend figures for ratios). Each transfection experiment was done in triplicate. 48 hours after transfection, cells were washed with PBS and harvested with 100 μl of lysis buffer (5 mM DTT, 250 mM Tris-HCl pH 8.1 containing protease inhibitors). A total of 100 μg of total cell lysate was incubating with 800 μl substrate solution (2 mg/ml ONPG (Pierce) in 0.1 M Na₂HPO₄, 1 M NaH₂PO₄, 1 M KCl, 1 M MgCl₂ with 20% β-mercaptoethanol) for 40 minutes at 37°C. The reaction was stopped by addition of 1 M Na₂CO₃ and the absorbance was measured at 420 nm.

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Supplementary Table S1. Human IKK α promoter analysis

Family matrix	Further information	Sequence (red:ci-value > 60); Capitals: core sequence	Optimal	Position from the TSS		Strand	Core Similarity	Matrix Similarity
<u>V\$RARF/RAR.01</u>	Retinoic acid receptor	tttgccagcacagta GTT Cattgtgagaa	0.97	-968	-940	(-)	0.938	0.908
<u>V\$E2FF/E2F.02</u>	E2F	aagct ctgc GAAAtcccg	0.84	-890	-874	(+)	1	0.886
<u>V\$GATA/GATA1.02</u>	GATA-binding factor 1	tcgg GATT tcgcg	1	-885	-873	(-)	1	0.921
<u>V\$E2FF/E2F.02</u>	E2F	aat ccga GAAAcactc	0.84	-880	-864	(+)	1	0.806
<u>V\$AP1F/AP1.01</u>	AP1	cttg AGT Catt	0.94	-839	-829	(+)	1	0.974
<u>V\$GATA/GATA3.01</u>	GATA-binding factor 3	ttgg GAT Gacagg	0.91	-829	-817	(+)	0.805	0.854
<u>V\$NFKB/NFKAPPAB.01</u>	NF-kappaB	ag GGG Atatcctg	0.89	-819	-807	(+)	1	0.864
<u>V\$EBOR/DELTAEF1.01</u>	deltaEF1	gagc AGG Tgtatt	0.99	-806	-794	(+)	1	0.959
<u>V\$CREB/CJUN_ATF2.01</u>	c-Jun/ATF2 heterodimers	gttcac TGAC Ttccaataca	0.99	-799	-779	(-)	1	0.94
<u>V\$OCTB/TST1.01</u>	POU-factor Tst-1/Oct-6	atga ATTA gcctt	0.9	-775	-763	(-)	1	0.905
<u>V\$GATA/GATA1.02</u>	GATA-binding factor 1	caat GATT taaag	1	-722	-710	(-)	1	0.91
<u>V\$GATA/GATA3.02</u>	GATA-binding factor 3	gat GAT ggcagg	0.91	-667	-655	(+)	1	0.842
<u>V\$GATA/GATA1.02</u>	GATA-binding factor 1	taac GATT tcgca	1	-737	-725	(+)	1	0.914
<u>V\$WHZF/WHN.01</u>	Winged helix protein	ggc ACG Ctaaa	0.95	-703	-693	(-)	1	0.942
<u>V\$GATA/GATA1.01</u>	GATA-binding factor 1	ggag GATA gatgg	0.96	-671	-659	(+)	1	0.923
<u>V\$GATA/GATA3.02</u>	GATA-binding factor 3	cag GAT ctgaag	0.91	-658	-646	(+)	0.812	0.895
<u>V\$GATA/GATA1.01</u>	GATA-binding factor 1	tgta GATA ggagt	0.96	-635	-623	(+)	1	0.979
<u>V\$GATA/GATA1.02</u>	GATA-binding factor 1	ctgg GATT ataga	1	-570	-558	(-)	1	0.989
<u>V\$NFKB/NFKAPPAB.01</u>	NF-kappaB	tc GGG Accagcct	0.89	-513	-501	(+)	1	0.839
<u>V\$SMAD/SMAD3.01</u>	Smad3	GTCT tgcta	0.99	-492	-484	(-)	1	0.914
<u>V\$E2FF/E2F.03</u>	E2F	gcag GCG Cgaccacct	0.85	-448	-432	(-)	1	0.914
<u>V\$CHOP/CHOP.01</u>	CHOP and C/EBPalpha	gcct GCA Atccca	0.9	-437	-425	(+)	1	0.924
<u>V\$GATA/GATA1.02</u>	GATA-binding factor 1	ctgg GATT gcagg	1	-436	-424	(-)	1	0.948
<u>V\$EBOR/DELTAEF1.01</u>	deltaEF1	cggg AGG Tgagaa	0.99	-418	-406	(+)	1	0.959
<u>V\$CHOP/CHOP.01</u>	CHOP and C/EBPalpha	gagt GCA Atggca	0.9	-360	-348	(-)	1	0.905
<u>V\$SMAD/SMAD3.01</u>	Smad3	GTCT cgctc	0.99	-333	-325	(-)	1	0.923
<u>V\$OCT1/OCT1.05</u>	Octamer-binding factor 1	cc CAT Cttaatataa	0.89	-324	-310	(+)	0.95	0.903
<u>V\$OCTB/TST1.01</u>	POU-factor Tst-1/Oct-6	tta ATTA agatg	0.9	-322	-310	(-)	1	0.93
<u>V\$OCTB/TST1.01</u>	POU-factor Tst-1/Oct-6	ctta ATTA aaaag	0.9	-319	-307	(+)	1	0.906
<u>V\$LEFF/LEF1.02</u>	TCF/LEF-1	cacttt CAA Agcagcc	0.94	-285	-269	(-)	1	0.968
<u>V\$GATA/GATA1.02</u>	GATA-binding factor 1	agtg GATT tgacg	1	-272	-260	(+)	1	0.919
<u>V\$GATA/GATA3.02</u>	GATA-binding factor 3	gag AGAT cctatg	0.91	-244	-232	(+)	1	0.991
<u>V\$SMAD/SMAD3.01</u>	Smad3	GTCT tgjaa	0.99	-229	-221	(-)	1	0.906
<u>V\$STAT/STAT5.01</u>	STAT5	tgtt TCC Aagactagaaa	0.89	-233	-215	(+)	0.945	0.816
<u>V\$NFKB/NFKAPPAB.01</u>	NF-kappaB	ta GGG Aagggcct	0.89	-93	-81	(+)	1	0.855
<u>V\$ETSF/CETS1P54.01</u>	c-Ets-1(p54)	ggtta CCG Gaagtgacg	0.92	-71	-55	(+)	1	0.997
<u>V\$TCFF/TCF11.01</u>	TCF11/KCR-F1/Nrf1	GTC Actt	1	-62	-56	(-)	1	0.9
<u>V\$LEFF/LEF1.01</u>	TCF/LEF-1	gcgagaa CAA Agacgcg	0.86	-43	-27	(+)	1	0.911
<u>V\$E2FF/E2F.03</u>	E2F	agac GCG Cgagcatcgg	0.85	-33	-17	(+)	1	0.804
<u>V\$E2FF/E2F.03</u>	E2F	ggcg GCC Cgaaaccggc	0.85	-18	-2	(+)	0.809	0.873