Isoforms of ΔNp63 and the migration of ocular limbal cells in human corneal regeneration

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The *p63* gene generates transactivating and N-terminally truncated transcripts (ΔNp63) initiated by different promoters. Alternative splicing gives rise to three different C termini, designated α, β, and γ. In the ocular epithelium, the corneal stem cells, which are segregated in the basal layer of the limbus, contain the α isoform but not β or γ. Holoclones derived from the limbus are rich in α, meroclones contain little, and paraclones contain none. In normal resting corneal epithelium, p63 of all isoforms is absent. Upon corneal wounding, cells originating from the limbus and containing α migrate progressively through the epithelium of the peripheral and central cornea. In the absence of an attached limbus, no α isoform appears in the corneal epithelium. When migrating cells containing the α isoform appear in the wounded corneal epithelium, they are confined to the basal layer, but the suprabasal cells, not only of the cornea but of the limbus as well, contain mRNA encoding β and γ. These data support the concept that the α isoform of p63 is necessary for the maintenance of the proliferative potential of limbal stem cells and their ability to migrate over the cornea. The β and γ isoforms, being suprabasal and virtually absent from the resting limbus, are not stem cell markers but are likely to play a role in epithelial differentiation specifically during the process of corneal regeneration.

limbus | stem cell

Keratinocyte stem cells govern the renewal of squamous epithelia by generating transient amplifying cells (TA cells) that terminally differentiate after a limited number of cell divisions (1–4). Human keratinocyte stem and TA cells, when isolated in culture, generate holoclones and paraclones, respectively (5–7).

The corneal epithelium provides an ideal experimental system in which to distinguish keratinocyte stem and TA cells (8). Corneal stem cells are segregated in the basal layer of the limbus, which is the zone encircling the cornea and separating it from the bulbar conjunctiva. TA cells that migrate from the limbus form the corneal epithelium (8). That the limbus is the site of stem cell precursors of the corneal epithelium is clear for several reasons: (*i*) the basal layer of the limbus lacks keratin 3 (a marker for corneal differentiation), whereas limbal suprabasal layers and all layers of the corneal epithelium express keratin 3 (9); (*ii*) the limbus contains slow-cycling cells and holoclone-forming cells, but the corneal epithelium does not (7, 10); (*iii*) the corneal epithelial cells are not self-sustaining; they divide only a few times during their migration from the limbus to the central cornea (11); (*iv*) restoration of destroyed limbal/corneal epithelium requires limbal transplantation (12) or grafts of autologous limbal cultures (13–15).

The gene with the most striking effects on the development of stratified epithelia is *p63* (16–19). Ablation of the *p63* gene in mice results in the absence of these epithelia (17, 18). In humans, mutations of the *p63* gene cause disorders of the epithelia and of nonepithelial structures whose development depends on the epithelial functions (20). The *p63* gene generates six isoforms (21). The transactivating isoforms are generated by the activity of an upstream promoter; the ΔN isoforms are produced from a downstream intronic promoter and lack the transactivation domain. For both transcripts, alternative splicing gives rise to three different C termini, designated α, β, and γ. ΔNp63 sustains the keratinocyte proliferative potential (22) that is characteristic of stem cells. Yet, the expression of p63 by the majority of basal cells and by suprabasal cells, as assessed by the 4A4 antibody recognizing all p63 isoforms, has been considered too broad to be stem cell specific (8, 23).

In this paper, we show that, depending on the conditions, limbal and corneal keratinocytes may contain all three ΔN isoforms. In the uninjured surface of the eye, ΔNp63α is present in the limbus but absent from the corneal epithelium. ΔNp63β and ΔNp63γ appear upon wounding and correlate with limbal cell migration and corneal regeneration and differentiation.

Materials and Methods

Human Specimens. Corneas taken (after permission) from organ donors were examined with a slit lamp immediately after retrieval and divided into two groups: (*i*) resting corneas, which did not show epithelial defect, dehydration, edema, or inflammation; and (*ii*) activated corneas, which had central corneal epithelial defects and/or abrasions, usually due to epithelial dehydration because of incomplete closure of the eyelids after death. Resting and activated corneas were taken 3.93 ± 0.69 and 6.79 ± 2.9 h from death, respectively. Unless otherwise indicated, half of each cornea was fixed in 4% paraformaldehyde (overnight at 4°C), and the other half was used for cell culture.

Cell and Tissue Culture. Keratinocytes were cultivated on a feeder layer of lethally irradiated 3T3-J2 cells (a gift from Howard Green, Harvard Medical School, Boston), as described (7). Colony-forming efficiency assays and calculation of the number of cell generations were performed as described (7). In some experiments, 100 limbal cells obtained from primary limbal cultures were plated in 100-cm dishes and cultured for 1 week. Colonies were then fixed in paraformaldehyde (4% in PBS for 10 min at room temperature) and subjected to immunofluorescence. For tissue culture experiments, corneas were incubated at 37°C (5% CO_2) in keratinocyte culture medium. In selected experiments, central corneas and limbal rings were surgically separated with the aid of a 5-mm corneal trephine. 3T3-J2 cells (5×10^5) were transfected with cDNA (8 μg) encoding ΔNp63 isoforms, by using Lipofectamine 2000 (Invitrogen).

Immunofluorescence and Western Analysis. Paraformaldehyde-fixed samples were embedded in OCT compound, frozen, and sectioned. Five- to 7-μm sections were analyzed by indirect immunofluorescence by using the 4A4 pan-p63 mAb [1:100, a gift from Frank McKeon (Harvard Medical School, Boston) or

Abbreviation: TA cells, transient amplifying cells.

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Table 1. Sequences for primers, annealing temperatures, and estimated length of PCR products

Primers	Sequence	Annealing temperature, °C	Product	Length, bp
TA	TGTTCAGTTCAGCCCATTGA	60	TAα	1,484
	5′ for TAp63α, TAp63β andTAp63γ	60	TAβ	1,471
		60	TAγ	1,264
ΔN	GGAAAACAATGCCCAGACTC	60	ΔNα	1,389
	5′ for ΔNp63α, ΔNp63β andΔNp63γ	60	ΔNβ	1,376
		60	ΔNγ	1,168
α	ATGATGAACAGCCCAACCTC			
	3′ for TAp63α andΔNp63α			
β	CAGACTTGCCAGATCCTGA			
	3′ for TAp63β andΔNp63β			
γ	GGGTACACTGATCGGTTTGG			
	3′ for TAp63γ andΔNp63γ			
β-Actin	5′GAGCGCAAGTACTCCGTGT	58		548
	3′ACGAAGGCTCATCATTCAAA			

purchased from BD Biosciences] and a p63α-specific antiserum. To generate such antiserum, rabbits were immunized with a synthetic peptide, NH_2, DFNFDMDARRNKQQRIKEEGE-COOH, comprising the C terminus postSAM domain of p63α (PRIMM, Milan). Purified IgG were used at a dilution of 1:200. Fluorescence-conjugated secondary antibodies were from Santa Cruz Biotechnology. Confocal and Z-stack analyses were done with the LSM510 Confocal Analyzer (Zeiss).

Western analysis was performed on cell extracts as described (24) by using 4A4 mAb (1:500) and p63α-specific IgG (1:500).

Semiquantitative RT-PCR. Total RNAs were purified with the RNase Micro Kit (Qiagen, Valencia, CA) and quantified by spectrophotometer analysis. RT-PCR was performed by using the OneStep RT-PCR Kit (Qiagen). cDNAs were synthesized from 0.5–2 μg of total RNA and PCR reactions carried out by

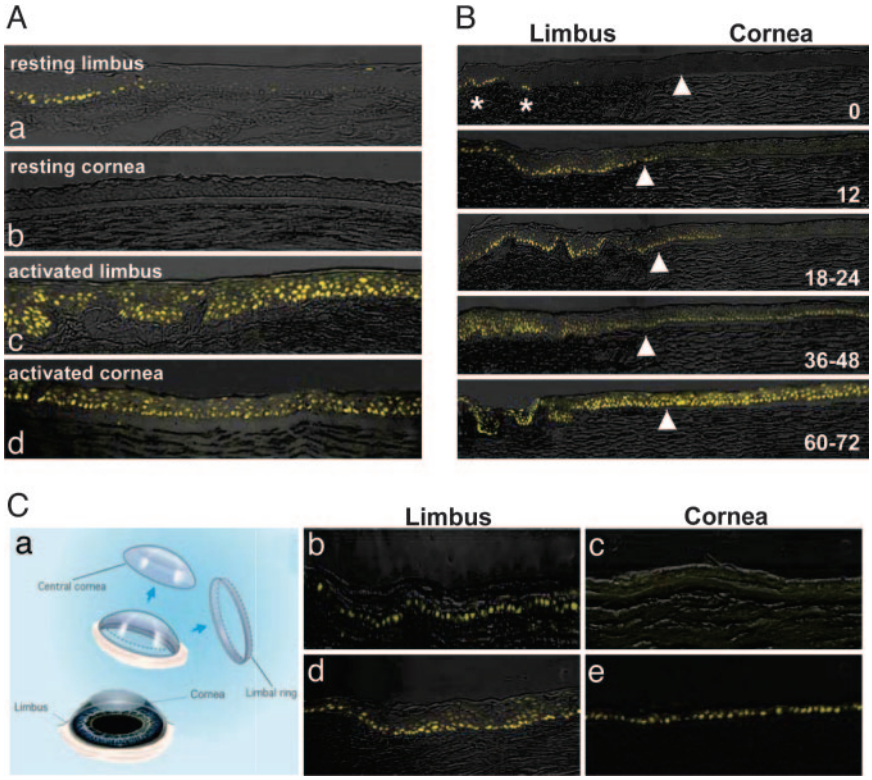

Fig. 1. Expression of p63 in resting and activated corneas. (*A*) 4A4 mAb immunofluorescent staining of resting (*a* and *b*), activated (*c* and *d*), limbal (*a* and *c*), and corneal (*b* and *d*) epithelium. (*B*) Progressive appearance of p63 in corneal epithelium. Resting corneas were placed in tissue culture after retrieval from donors. At different incubation time (hours indicated in each frame), specimens were immunostained with 4A4 mAb. Arrows indicate the limbus–cornea border. At time 0, clusters of $p63^+$ cells were observed only in the limbal basal layer (asterisks). At 12 h, the entire limbal basal layer expressed p63. Note the progressive appearance of $p63^+$ cells from the peripheral cornea to the central cornea (24–72 h). (*C*) p63 appears in the corneal epithelium as a result of cell migration from the limbus. The central cornea and the limbal ring of resting corneas were surgically separated with a 5-mm corneal trephine immediately after retrieval (*a*) and placed in tissue culture for 5 days. Samples were then immunostained with 4A4 mAb. Note that $p63^+$ cells were present in the limbal ring (*b*) but not in the separated central cornea (*c*). When the two were not separated, the cornea (e), like its associated limbus (*d*), expressed p63.

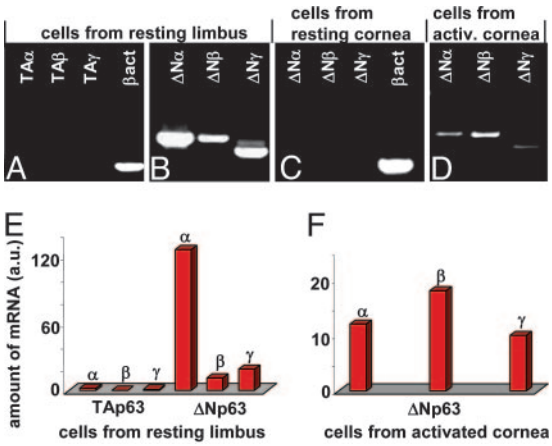

Fig. 2. RT-PCR analysis in cells cultured from resting and activated limbus and cornea. Semiquantitative RT-PCR analysis was performed by using primers specific for each of the p63 isoforms (Table 1). All three isoforms of ΔNp63 are present in cells cultured from the resting limbus but appear in the cornea only after activation. *E* and *F* show quantitation of PCR products shown in *A*, *B*, and *D*, respectively. β-Actin (βact) has been used as a control. Note the absence of all TAp63 isoforms.

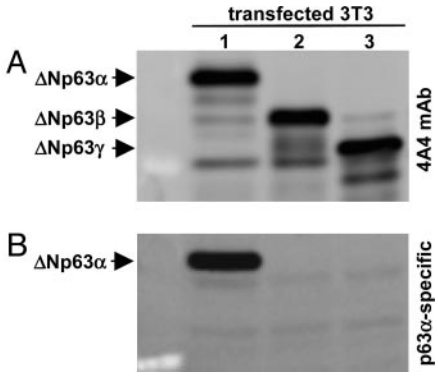

Fig. 4. Specificity of the antiserum raised against p63α. Parallel cultures of 3T3 cells were transfected with cDNA encoding either ΔNp63α (lanes 1), ΔNp63β (lanes 2), or ΔNp63γ (lanes 3). Cell extracts prepared from transduced 3T3 cells were fractionated on 7.5% SDS-polyacrylamide gels, transferred to poly(vinylidene difluoride) filters, and immunostained. 4A4 mAb (*A*) detects all three isoforms, whereas or p63α-specific IgG detects only the α isoform (*B*).

using 20, 24, 28, 32, 36, and 40 cycles. β-Actin was used for normalization. Ethidium bromide-stained agarose gels were visualized with Kodak Image Station 440 CF. Quantification was performed by using the software KODAK 1D 3,5. Band intensity was expressed as relative absorbance units. Primer sequences, annealing temperatures, and PCR product length are shown in Table 1.

***In Situ* Hybridization.** Digoxigenin-labeled cRNAs were synthesized according to the manufacturer's instructions (DIG RNA Labeling kit, Roche, Basel, Switzerland), and hybridization was performed as described (25). Primer pairs with Sp6/T7 promoter sequences (MWG Biotech,Ebersberg, Germany) were used to obtain DNA templates for *in vitro* transcription. The following primer sequences were used: ΔNp63α: 5′-Sp6-GAGGTTGGGCTGTTCATCAT-3′ [melting temperature (T_m) 60°C] and 5′-T7-GTGGGAAAGAGATGGTCTGG-3′ (T_m 58°C); ΔNp63β: 5′-Sp6-GGCTGGAGACATGAATG-GAC-3′ (T_m 60°C) and 5′-T7-CAGACTTGCCAGATC-CTGA-3′ (T_m 58°C); and ΔNp63γ: 5′-Sp6-ACGAAGATC-CCCAGATGATG-3′ (T_m 60°C) and 5′-T7-GGGTACACT-GATCGGTTTGG-3′ (T_m 58°C).

ΔNp63α, ΔNp63β and ΔNp63γ yielded an amplification product of 299, 132, and 286 bp, respectively. Sense riboprobes were used as negative controls.

Results

Normal unperturbated corneas (referred to as resting corneas) and wounded corneas (referred to as activated corneas) were used (see *Materials and Methods*). Data have been generated in experiments performed on 7 resting and 17 activated human corneas. ΔNp63α, ΔNp63β, and ΔNp63γ are hereafter referred to as α, β, and γ, respectively.

Expression of p63 in Resting and Activated Corneas. As shown in Fig. 1*A*, immunofluorescence performed on cryosections of resting corneas with 4A4 mAb (recognizing all p63 isoforms) revealed that p63 was present in patches of limbal basal cells (Fig. 1*Aa*) but was undetectable in the corneal epithelium (Fig. 1*Ab*). In contrast, 4A4 mAb decorated the entire basal layer and most of the suprabasal cells of limbal (Fig. 1*Ac*) and corneal (Fig. 1*Ad*) epithelia of activated corneas.

Semiquantitative RT-PCR was performed by using primers specific for each of the p63 isoforms (Table 1). mRNAs encoding all ΔNp63 isoforms were present in keratinocytes cultured from the resting limbus (Fig. 2*B*), but none were detectable in cells cultured from the resting cornea (Fig. 2*C*). In primary limbal cultures, mRNA encoding α was 11- and 7-fold higher than that of β and γ, respectively (Fig. 2*E*). In contrast, activated corneal cells contained β and γ (Fig. 2*D*), whose aggregate amount was

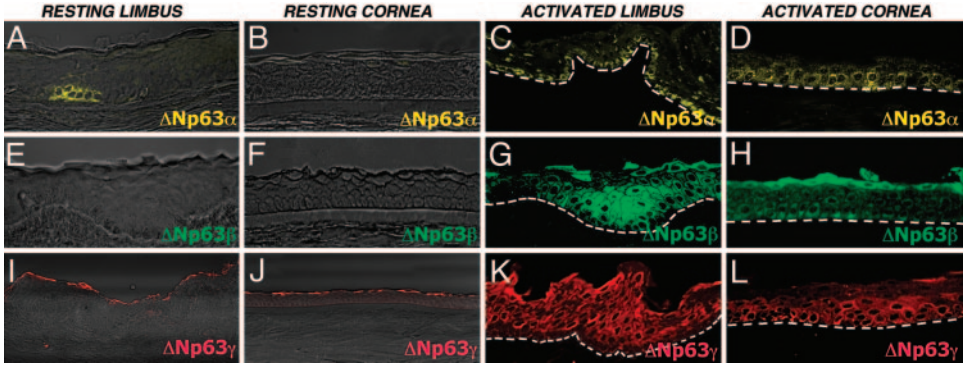

Fig. 3. Cytological detection of ΔNp63α, -β, and -γ transcripts in resting vs. activated limbus and cornea. Sections were processed for *in situ* hybridization with antisense digoxigenin-labeled cRNAs specific for each of the ΔNp63 isoforms. Note the abundant expression of β and γ transcripts in activated but not in resting epithelia. The dotted line underlines the basal layer of activated limbal and corneal epithelia.

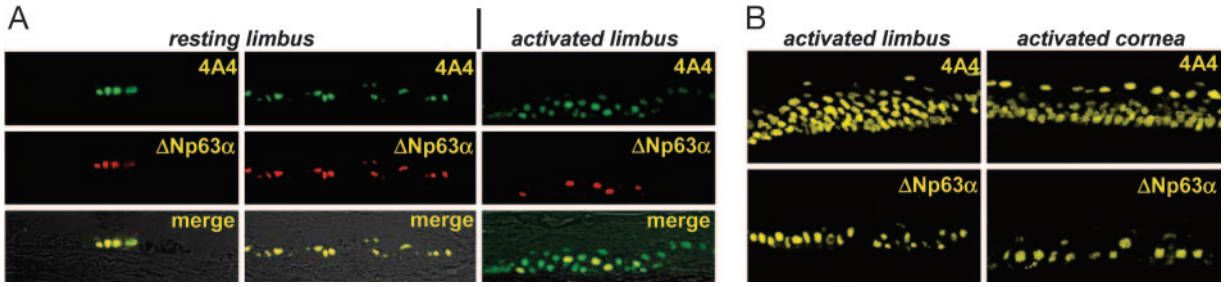

Fig. 5. Immunodetection of ΔNp63α in resting and activated limbus and cornea. (*A*) Double immunofluorescence of sections of resting and activated limbus stained with 4A4 mAb (green) and p63α-specific IgG (red). The yellow color in the ''merge'' frames indicates cells stained with both antibodies. Cells stained green alone express β and γ ΔNp63 but not the α isoform. The two series of frames indicated as resting limbus are from different experiments representative of the lowest (*Left*) and highest (*Right*) number of α-expressing cells, respectively. (*B*) Z-stack analysis of 6-μm sections of activated limbus and cornea immunostained with 4A4 mAb and α-specific IgG. Images were obtained with a LSM510 confocal microscope (Zeiss) and Z-stack analyzed with the LSM510 Analyzer. Most suprabasal cells express some isoforms of ΔNp63 but not α.

double that of α (Fig. 2*F*). We did not detect significant levels of transactivating p63 PCR products in cells of either resting (Fig. 2 *A* and *E*) or activated (not shown) corneas.

In situ hybridization showed that α mRNA was present in discrete patches of basal cells of the resting limbus (Fig. 3*A*) but was undetectable in suprabasal cells (Fig. 3*A*) and in the entire resting corneal epithelium (Fig. 3*B*). In resting limbal and corneal epithelia, we were unable to detect significant levels of β mRNA (Fig. 3 *E* and *F*), whereas tiny amounts of γ mRNA were present in the uppermost layers of both epithelia (Fig. 3 *I* and *J*). In sharp contrast, activated limbal and corneal epithelia contained mRNA encoding all ΔN isoforms (Fig. 3). α mRNA were present only in the basal layer of the activated epithelia (Fig. 3 *C* and *D*). Abundant β and γ mRNAs were confined mainly to suprabasal layers of the activated limbus (Fig. 3 *G* and *K*) but were present in all cell layers of the activated corneal epithelium (Fig. 3 *H* and *L*). Comparable sections were stained with 4A4 mAbs and p63α-specific IgG (Fig. 5).

To distinguish α from the other isoforms, we generated a rabbit antiserum against the α isoform-specific postSAM domain (see *Materials and Methods*). To assess the specificity of the antiserum, parallel cultures of 3T3 cells were transfected with cDNA encoding each of the ΔNp63 isoforms and subjected to Western analysis. As shown in Fig. 4, 4A4 mAb recognized all ΔNp63 isoforms in cell exctracts prepared from transfected 3T3 cells (Fig. 4*A*, lanes 1–3), whereas the IgG fraction of the newly generated antiserum recognized only the α isoform (Fig. 4*B*, lanes 1–3). The staining was abolished by the addition of 10 μg of the synthetic peptide used to raise the antiserum but not by an equivalent amount of a control peptide (not shown). Because we could not detect transactivating p63 in human corneas, we conclude that the α isoform detected in our samples by the newly generated antiserum was present only in ΔNp63.

Double immunofluorescence was performed on resting and activated limbal epithelium. In resting limbus, 4A4 mAb and α-specific IgG identified the same patches of cells located in the basal layer (Fig. 5*A*). In contrast, in activated limbus, α-specific IgG identified only a subset of the many basal and suprabasal cells stained by 4A4 mAb (Fig. 5*A*). The nuclei of limbal cells were stained with DAPI, and the proportion of α-positive cells was calculated. One millimeter of resting limbal epithelium contained ≈150 basal cells, of which an average of 13 were α-positive. Thus, α was present in ≈8% of limbal basal cells. Estimates of stem cell numbers from *in vivo* (11) and *in vitro* (7) studies have indicated that stem cells constitute between 1% and 10% of the limbal basal layer. In 1 mm of activated limbal epithelium, we found an average of 55 α-positive cells, or 36% of basal cells.

The different distribution of ΔNp63 isoforms was confirmed by immunofluorescence followed by confocal microscopy and Z-stack analysis of activated limbal and corneal epithelia (Fig. 5*B*); α was expressed mainly, if not exclusively, in some limbal and corneal basal cells. Thus, part of the basal and most of the suprabasal staining observed with 4A4 mAb in activated limbal and corneal epithelia should be ascribed to the β and γ isoforms.

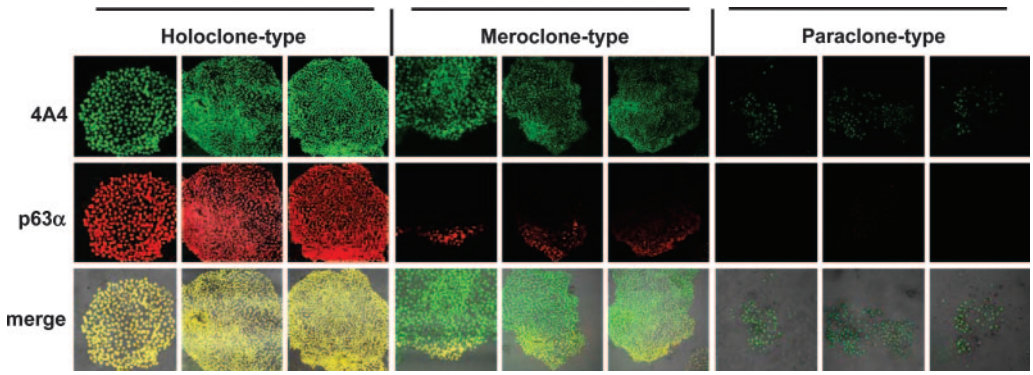

Fig. 6. Identification of holoclones by their content of ΔNp63α. One hundred cells obtained from primary limbal cultures were plated in 100-cm dishes and cultured for 1 week. Colonies were then examined under an Axiovert 200 M microscope (Zeiss); large round colonies with smooth and regular borders and formed entirely by small cells with scarce cytoplasm were classified as holoclones. Large colonies formed by small cells but showing irregular borders and/or with areas containing large cells were classified as meroclones. Small colonies with wrinkled and irregular borders and formed by large cells were classified as paraclones. Double immunofluorescence was performed on paraformaldehyde-fixed colonies with 4A4 mAb (green) and p63α-specific IgG (red). The yellow color in the ''merge'' frames indicates cells stained with both antibodies. The three clonal types are most clearly distinguished by the presence or the absence of the α isoform.

These data are consistent with the distribution of ΔNp63 mRNA shown in Fig. 3.

Expression of ΔNp63α in Limbal Clonal Types. Three clonal types of keratinocytes, known as holoclones, meroclones, and paraclones, have been identified in cultures of human squamous epithelia (5–7). The holoclone has the properties of a keratinocyte stem cell (5–7, 24, 26–29). The paraclone is generated by a TA cell, and the meroclone is an intermediate type that gives rise to paraclones (5–7). We have previously shown that holoclones contain abundant p63, whereas paraclones contain abundant markers of terminal differentiation (24, 27).

Double immunofluorescence was performed on the different types of clones isolated from primary limbal cultures by using 4A4 mAb and α-specific IgG. As shown in Fig. 6, all cells of colonies produced by holoclones contained α. In contrast, cells of colonies formed by paraclones lack α. Because the cells of paraclones are stained by the 4A4 mAb (Fig. 6), this staining must be due to β and γ. Cells of colonies formed by meroclones were rather well stained by 4A4 but very much less by the antibody to α, which stained only the leading edge of the colonies (Fig. 6). Their 4A4 staining must be mostly due to β and γ. It is concluded that the isoform of ΔNp63 that most precisely characterizes clonal types is the α isoform.

Migration of p63$^+$ Cells from the Limbus to the Central Cornea. In preliminary experiments, we observed that when a resting cornea was placed in culture medium, p63 appeared in the corneal epithelium, mimicking the activation process induced by corneal damage. To investigate whether this was because of *de novo* expression of p63 in corneal cells or migration of limbal cells containing p63 to the cornea, we placed resting corneas in tissue culture and analyzed the appearance of p63 with time by using the 4A4 mAb. At time 0, p63 was present only in patches of limbal basal cells, as expected (Fig. 1*B*, asterisks). With time, we observed the appearance of p63$^+$ cells, first in the peripheral cornea (Fig. 1*B*, 12–24 h) and then in the central cornea (Fig. 1*B*, 36–48 h). By 60–72 h, p63 was clearly detected in suprabasal cells as well. This pattern suggests that p63$^+$ limbal cells migrate to the central cornea upon activation.

To strengthen this observation, we performed an experiment in which the central part of the resting cornea and its limbal ring were surgically separated at retrieval from the donor, placed in tissue culture for 5 days, and then analyzed by immunofluorescence (Fig. 1*Ca*). As shown in Fig. 1*Cc*, no p63 staining was observed in the separated corneal epithelium. In contrast, the control cornea that maintained contact with the limbus expressed p63, as expected (Fig. 1*Ce*). The limbal staining was not affected by the separation (Fig. 1*C b* and *d*). It is therefore clear that the appearance of p63 in corneal cells was because of migration of limbal cells containing p63.

If limbal cells migrate to the central cornea, one would expect that corneal epithelial might acquire clonogenic and proliferative capabilities similar to those of limbal cells. Resting limbal cells generated large and smooth colonies (Fig. 7*A*), whereas the corresponding corneal cells generated only few aborted colonies (Fig. 7*B*). The cultured limbal cells underwent 85 cell doublings before senescence (Fig. 7*E*, yellow bar), whereas the few colonies generated by corneal cells (Fig. 7*B*) could not be passaged (Fig. 7*E*, blue bar). In contrast, limbal and corneal epithelial cells obtained from activated corneas both generated large smooth colonies with a similar efficiency (Fig. 7 *C* and *D*, respectively) and underwent ≈100 cell doublings before senescence (Fig. 7*E*, red and green bars).

When keratinocytes are placed in culture, they proliferate to cover the surface vessel, hence they mimic the activation process observed *in vivo* upon wounding. With respect to the ΔNp63 isoforms, α, β, and γ were detected in limbal but not in corneal cells cultivated from resting corneas (Fig. 7*F*). In contrast, α, β, and γ were detected in both limbal and corneal cells cultured from activated corneas (Fig. 7*F*). This shows that resting corneal cells upon cultivation are unable to acquire any ΔNp63 isoform, and that only by migration of activated limbal cells can the corneal epithelium acquire p63.

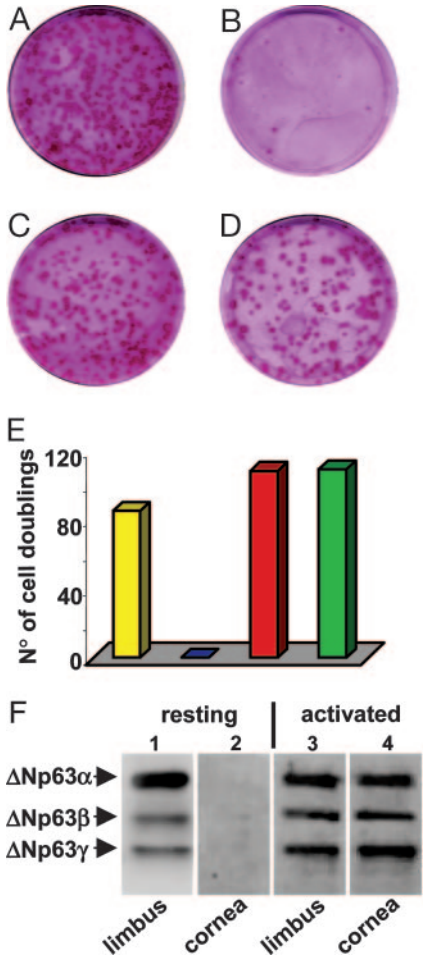

Fig. 7. Clonogenic and proliferative capacities and p63 content of cells cultured from resting and activated limbus and cornea. (*A–D*) Clonogenic ability. One thousand keratinocytes obtained from primary cultures established from resting and activated limbus and cornea were cultivated for 12 days and stained with Rhodamine B. Although resting corneal cells generated only few aborted colonies (*B*), resting limbal cells (*A*) and activated limbal (*C*) and corneal (*D*) cells had a comparable clonogenic ability. (*E*) Proliferative capacity. Keratinocytes were then serially cultivated, and the number of cell generations was calculated. Although resting corneal cells could not be passaged (blue), resting limbal (yellow), activated limbal (red), and activated corneal cells (green) underwent a comparable number of cell doublings. (*F*) p63 content by Western analysis. Cell extracts were prepared from limbal (lanes 1 and 3) and corneal (lanes 2 and 4) cultures established from resting (lanes 1 and 2) and activated (lanes 3 and 4) corneas. Equal amounts of protein were fractionated on 7.5% SDS-polyacrylamide gels, transferred to poly(vinylidene difluoride) filters, and immunostained with 4A4 mAb. ΔNp63α, β, and γ were detected in limbal but not in corneal cells cultivated from resting corneas and in both limbal and corneal cells cultured from activated corneas.

Discussion

In the resting ocular epithelium, the α isoform of ΔNp63 is present only in the basal layer of the limbus. The following evidence shows that ΔNp63α is likely to identify the stem cell population of the human limbus and supports the concept that α is the isoform of ΔNp63 essential for regenerative proliferation

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in the ocular surface: (*i*) the location of discrete clusters of α^+ cells in the limbal basal layer (where slow-cycling cells are located), (*ii*) the number of α^+ cells ($\approx$8% of basal cells), and (*iii*) the abundant α in holoclones, the sharply reduced amount in meroclones, and its absence from paraclones. The number of α^+ limbal cells was significantly higher in wounded corneas, suggesting that human limbal stem cells divide upon corneal injury. Similarly, large numbers of murine slow-cycling limbal cells have been shown to proliferate upon removal of a portion of the central corneal epithelium (11).

Corneal regeneration is accompanied by the appearance of the β and γ isoforms of ΔNp63. Neither β nor γ isoforms are present in substantial amounts in resting corneas, but both become abundant in activated limbal and corneal epithelia. Both appear upon cultivation of limbal cells. Paraclones contain β and γ but not α. The presence of β and γ in the suprabasal layers suggests they might regulate aspects of cell differentiation, specifically during the process of wound healing and corneal regeneration. This is consistent with the presence of one or both of these isoforms in TA cells (paraclones).

The presence of $p63^+$ cells in the activated central corneal epithelium is due to migration of $p63^+$ cells from the limbus. This explains why corneal cells cultivated from a resting cornea proliferate very little and do not express p63. Activated corneal cells, however, have the same clonogenic and proliferative capabilities possessed by their limbal counterparts. This clearly suggests that, upon corneal damage, some of the multiplying $p63^+$ limbal stem cells migrate to the central cornea, where they contribute to epithelial regeneration.

The expression of p63 isoforms in other stratified squamous epithelia and their role in stem cell determination and differentiation should be evaluated in light of the roles of the different p63 isoforms in the human limbus and cornea.

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