# Involvement of p27$^{KIP1}$ in the Proliferation of the Developing Corneal Endothelium

*Kazuhiko Yoshida,*[1] *Satoru Kase,*[1] *Keiko Nakayama,*[2] *Hiroyasu Nagahama,*[3] *Takayuki Harada,*[1] *Hiromi Ikeda,*[4] *Chikako Harada,*[1] *Junko Imaki,*[5] *Kazuhiro Ohgami,*[1] *Kenji Shiratori,*[1] *Iliyana Bozhidarova Ilieva,*[1] *Shigeaki Ohno,*[1] *Shinzo Nishi,*[4] *and Keiichi I. Nakayama*[3]

**Purpose.** To examine the involvement of p27$^{KIP1}$ in the regulation of the proliferation of the developing corneal endothelium.

**Methods.** Central and peripheral corneas in C57Bl6 mice at postnatal day (P)1, P11, and 12 weeks after birth were analyzed by immunocytochemistry with anti-p27$^{KIP1}$, -p57$^{KIP2}$, and -proliferating cell nuclear antigen (PCNA) antibodies. Nuclear staining was performed with 4′,6′-diamino-2-phenylindole (DAPI) in wholemounts of corneal endothelium of the center and peripheral cornea in wild-type and p27$^{KIP1}$ knockout (−/−) mice at 12 weeks of age. p27$^{KIP1-/-}$ and control mice were injected with bromodeoxyuridine (BrdU) once on P7, twice per day on P8 and P9, and once on P10 and then were analyzed by a BrdU cell-proliferation assay on P11.

**Results.** On P1, p27$^{KIP1}$ immunoreactivity was detected in a small number of corneal endothelial cells, and many endothelial cells expressed PCNA. At P11 and 12 weeks after birth, p27$^{KIP1}$ immunoreactivity was detected in many corneal endothelial cells. PCNA-positive cells in the endothelium were rare on P11 and completely absent at 12 weeks after birth. p57$^{KIP2}$ was not detected in either corneal epithelium or endothelium at P1, P11, or 12 weeks after birth. In wholemounts of corneal endothelium at 12 weeks of age, the number of endothelial nuclei in the p27$^{KIP1-/-}$ mice was significantly higher than that in wild-type mice in both the center and peripheral regions of the cornea. In the BrdU assay, positive cells were abundant in the corneal endothelium of p27$^{KIP1-/-}$ mice, whereas there were few positive cells in control mice. PCNA immunoreactivity in the endothelium of the p27$^{KIP1-/-}$ mice was completely absent at 12 weeks after birth.

**Conclusions.** These results suggest that p27$^{KIP1}$ is involved in the regulation of proliferation in the endothelium of the developing cornea. (*Invest Ophthalmol Vis Sci.* 2004;45:2163–2167) DOI:10.1167/iovs.03-1238

The corneal endothelium shows a monolayer of differentiated cells located in the posterior portion of the cornea. The capacity for regeneration of corneal endothelium after injury is severely limited in humans; thus, corneal endothelium is considered to be a nonreplicating tissue. The cellular mechanisms responsible for mitotic inhibition in the corneal endothelium are not clear but may be similar to the mechanisms that contribute to cessation of division in the newly formed endothelium of developing cornea. Early in corneal development, the putative endothelial cells actively divide to form the endothelium.[1] As the endothelial monolayer matures, the cells establish gap and tight junctions, and proliferation essentially ceases.

Cell cycle progression is controlled by a series of kinase complexes composed of cyclins and cyclin-dependent kinases (CDKs).[2] Enzymatic activities of cyclin/CDK complexes are regulated by many mechanisms that reflect both the diversity of the signals that they integrate and the central importance of their roles in cell cycle control. These regulatory mechanisms include the actions of CDK inhibitors (CKIs) such as p27$^{KIP1}$ and p57$^{KIP2}$.[3–5] In various cell lines, elimination of p27$^{KIP1}$ during the late $G_1$ phase has been shown to be required for cell cycle progression to the S phase.[6–9] Consistent with this idea is the fact that forced expression of p27$^{KIP1}$ blocks cell cycle progression during the $G_1$ phase, whereas targeted p27$^{KIP1}$ mRNA antisense vectors increase the fraction of cells in S phase.

A study has demonstrated that p27$^{KIP1}$ mRNA does fluctuate during the cell cycle, implying that control of p27$^{KIP1}$ expression is exerted posttranslationally.[10] The ubiquitin-mediated proteosome pathway has been suggested to be involved in p27$^{KIP1}$ degradation in mammals.[11] This proteosome pathway is emerging as a major universal mechanism that regulates selective and time-controlled elimination of key short-lived regulatory proteins, such as CKIs[12] and IκB.[13] In our recent paper, we suggested that this pathway is involved in the differentiation of corneal epithelial cells.[14] The ubiquitin-mediated pathway of protein degradation comprises two discrete steps: the covalent attachment of multiple ubiquitin molecules to the protein substrate, and degradation of the polyubiquitylated protein by the 26S proteosome complex. The Skp1-Cullin-F-Box protein (SCF) complex is one of the major classes of ubiquitin ligase and determines the specificity of protein ubiquitylation. Skp2 is an F-box protein that is a component of the SCF complex.[15]

It has been reported that p27$^{KIP1}$ is specifically recognized by Skp2 and that this step was a rate-limiting component of the machinery that ubiquitinated and degraded p27$^{KIP1}$.[16,17] We

From the Departments of [1]Ophthalmology and [4]Biochemistry, Hokkaido University School of Medicine, Sapporo, Japan; the [5]Department of Anatomy, Nippon Medical College, Tokyo, Japan; the [3]Departments of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; and the [2]Division of Developmental Genetics, Center for Translational and Advanced Animal Research on Human Diseases, Tohoku University Graduate School of Medicine, Miyagi, Japan.

Submitted for publication November 13, 2003; revised January 26, 2004; accepted February 13, 2004.

Disclosure: **K. Yoshida**, None; **S. Kase**, None; **K. Nakayama**, None; **H. Nagahama**, None; **T. Harada**, None; **H. Ikeda**, None; **C. Harada**, None; **J. Imaki**, None; **K. Ohgami**, None; **K. Shiratori**, None; **I.B. Ilieva**, None; **S. Ohno**, None; **S. Nishi**, None; **K.I. Nakayama**, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Kazuhiko Yoshida, Department of Ophthalmology, Hokkaido University School of Medicine, N17 W5, Kita-ku, Sapporo, 060-8638 Japan; kyoshida@med.hokudai.ac.jp.


Copyright © Association for Research in Vision and Ophthalmology


Downloaded from iovs.arvojournals.org on 01/25/2021

have reported that $p27^{KIP1}$ is expressed in corneal epithelium.[18] This expression was not detected 24 hours after epithelial scraping, when there were many cells in S phase of the cell cycle in the corneal epithelium. Twenty-four hours after epithelial scraping in $Skp2^{-/-}$ mice, the corneal epithelium was thinner than that in wild-type mice and had many $p27^{KIP1}$-positive cells and few cells in the S phase. These results suggest that degradation of $p27^{KIP1}$ by Skp2 is involved in the regulation of proliferation in response to wounding of the corneal epithelium.

In the present study, we analyzed the expression of $p27^{KIP1}$ as well as proliferating cell nuclear antigen (PCNA), one of the essential replication factors, which was induced at late $G_1$ phase and reached a peak level in the S phase of the cell cycle[19,20] in the developing cornea. In addition, the corneal endothelium of $p27^{KIP1}$ knockout mice were also examined.

## Materials and Methods

### Development of $p27^{KIP1}$ Knockout Mice

C57Bl6 mice were obtained from Hokudo Corp. (Sapporo, Japan). The $p27^{KIP1}$ knockout (−/−) and littermate control mice were obtained as previously reported.[21,22] Briefly, cloned genomic DNA corresponding to the $p27^{KIP1}$ loci was isolated from a 129/Sv mouse genomic library. To produce $p27^{KIP1-/-}$ mice, a targeting vector was constructed by replacing a 2.5-kb *Sma*I-*Sma*I fragment containing the entire $p27^{KIP1}$ sequence with a PGK-neo-poly(A) cassette. Maintenance, transfection, and selection of ES cells were performed as described previously.[21] Mutant ES cells were microinjected into C57Bl6 mouse embryos, and the resultant male chimeras were mated with female C57Bl6 mice.

### Immunocytochemistry

Eyes were dissected from C57Bl6 mice at postnatal day (P)1, P11, and 12 weeks after birth. Eyes were also dissected from $p27^{KIP1}$ knockout mice at P11 and 12 weeks after birth. For the bromodeoxyuridine (BrdU) assay, $p27^{KIP1-/-}$ mice and littermate controls were injected with BrdU (30 mg/kg) once on P7, twice per day on P8 and P9, and once on P10, and the eyes were dissected at P11. Dissected eyes were washed in saline and fixed in ice-cold 4% paraformaldehyde (PFA) in 0.1 M borate buffer (pH 9.5) for 2 hours, and processed for paraffin sectioning. The animal experiments conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Sections were dewaxed and rehydrated and then rinsed twice in phosphate-buffered saline (PBS) and incubated with normal goat serum and then with a 1:1000 dilution of mouse monoclonal anti-$p27^{KIP1}$ antibody (BD-Pharmingen, San Diego, CA), or anti-PCNA (1:1000; Zymed, South San Francisco, CA), or anti-$p57^{KIP2}$ antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA). Anti-BrdU staining was performed as described by Yoshiki et al.[23] Briefly, the sections were immersed in 0.4 mg/mL pepsin in 0.1 N HCl at 30°C for 1 minute and then in 2N HCl at 40°C for 1 hour. After the slides were washed with PBS, they were incubated with normal goat serum and then with anti-BrdU antibody (BD Biosciences, San Jose, CA) at a dilution of 1:1000. Binding of the primary antibody was localized by fluorescence microscopy using FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) at a dilution of 1:200.

### Corneal Endothelium Wholemount

Eyes were dissected from $p27^{KIP1-/-}$ and wild-type mice (12 weeks of age). The center and periphery of the cornea were removed and stained with 4′6-diamidino-2-phenylindole (DAPI; 10–4 μg/mL) in PBS for 5 minutes. The corneas then were laid flat on a glass slide with the endothelium facing up and examined with a microscope equipped for epifluorescence (Axiovert 35M; Carl Zeiss Meditec, Dublin, CA).

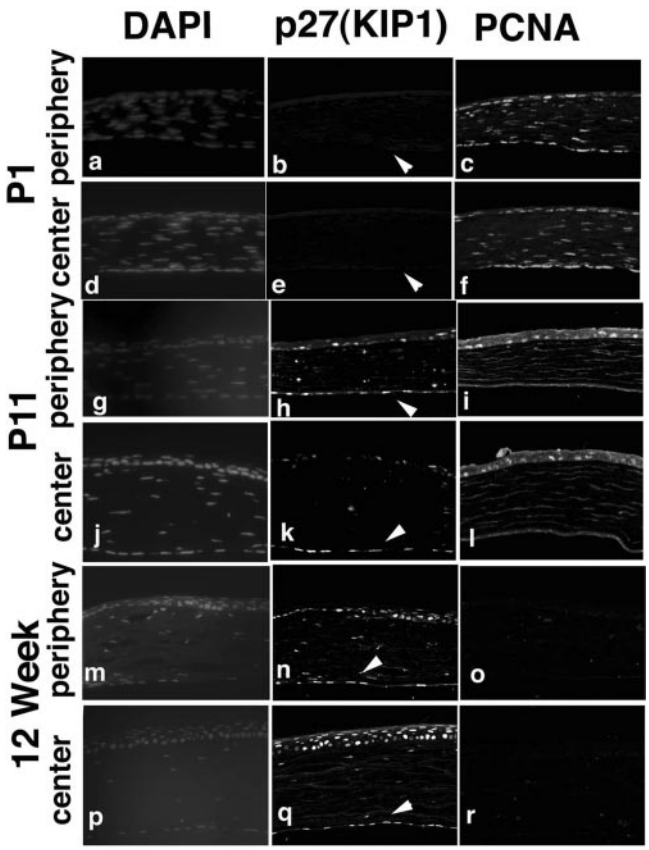


**Figure 1.** Nuclear staining with DAPI (**a**, **d**, **g**, **j**, **m**, **p**) and expression of $p27^{KIP1}$ (**b**, **e**, **h**, **k**, **n**, **q**) and PCNA (**c**, **f**, **i**, **l**, **o**, **r**) in the central (**d**–**f**, **j**–**l**, **p**–**r**) and peripheral (**a**–**c**, **g**–**i**, **m**–**o**) corneas of mice at P1 (**a**– **f**), P11 (**g**–**l**), or 12 weeks (**m**–**r**) after birth. $p27^{KIP1}$ was expressed in a small number of corneal endothelial cells (**b**, **e**, *arrowhead*), whereas many endothelial cells expressed PCNA on P1 (**c**, **f**). In contrast, many endothelial cells in the central and peripheral cornea showed nuclear immunoreactivity for $p27^{KIP1}$ at P11 and 12 weeks after birth (**h**, **k**, **n**, **q**; *arrowhead*). PCNA-positive cells in the endothelium were rare on P11 (**i**, **l**) and were completely absent at 12 weeks (**o**, **r**) after birth. Magnification: (**a**– **l**) ×630; (**m**– **r**) ×400 .

### Statistical Analysis

For the center of the cornea, a microscopic field of identical size (345.6 × 276.5 μm) was chosen at the center of each sample.[24] For the peripheral region, the same size of microscopic field was chosen so that the peripheral margin was 310 μm from the iris route. Seven corneas each from $p27^{KIP1-/-}$ mice and wild-type mice of each strain were used. The results were evaluated using the Aspin-Welch *t*-test.

## Results

In this study, the distribution of $p27^{KIP1}$ was examined in the corneas of mice at P1, P11, and 12 weeks after birth (Fig. 1). On P1, $p27^{KIP1}$ immunoreactivity was detected in a small number of corneal endothelial cells (Figs. 1b, 1e; arrowheads), and PCNA was detected in many endothelial cells (Figs. 1c, 1f). At P11 and 12 weeks after birth, $p27^{KIP1}$ immunoreactivity was detected in many corneal endothelial cells (Figs. 1h, 1k, 1n, 1q; arrowheads). PCNA-positive cells in the endothelium were rare on P11 and completely absent at 12 weeks after birth (Figs. 1i, 1l, 1o, 1r). We examined six animals at each stage and four sections per animal to determine the positive rate of $p27^{KIP1}$ staining in corneal cells and had successive results.

Downloaded from iovs.arvojournals.org on 01/25/2021

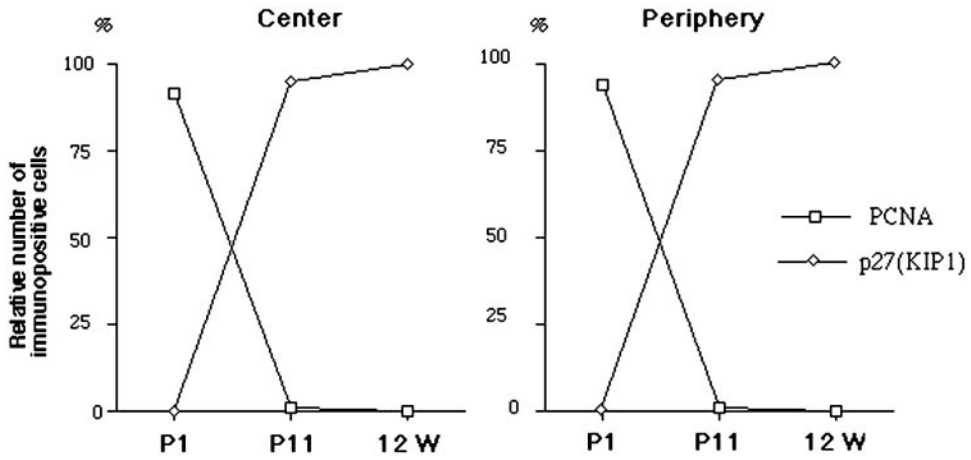


**FIGURE 2.** The number of cells positive for p27$^{KIP1}$ and PCNA relative to the number of nuclei stained with DAPI among the endothelial cells of the central (*left*) and peripheral (*right*) cornea at P1, P11, or 12 weeks (12w) after birth.

Figure 2 summarizes the number of cells positive for p27$^{KIP1}$ and PCNA among the endothelial cells of the central (left) and peripheral (right) cornea at P1, P11, and 12 weeks after birth. Expression of p27$^{KIP1}$ and PCNA appeared to be negatively correlated. p57$^{KIP2}$ immunoreactivity was detected in the equator of the P1 lens as previously described,[25] but was not detected in either the corneal epithelium or endothelium at P1, P11, or 12 weeks after birth (data not shown).

To examine the role of p27$^{KIP1}$ in the development of the corneal endothelium, nuclear staining with DAPI was performed with wholemounts of the endothelium of central and peripheral corneas. Figure 3 shows representative views of endothelium from 12-week-old wild-type and p27$^{KIP1-/-}$ mice.

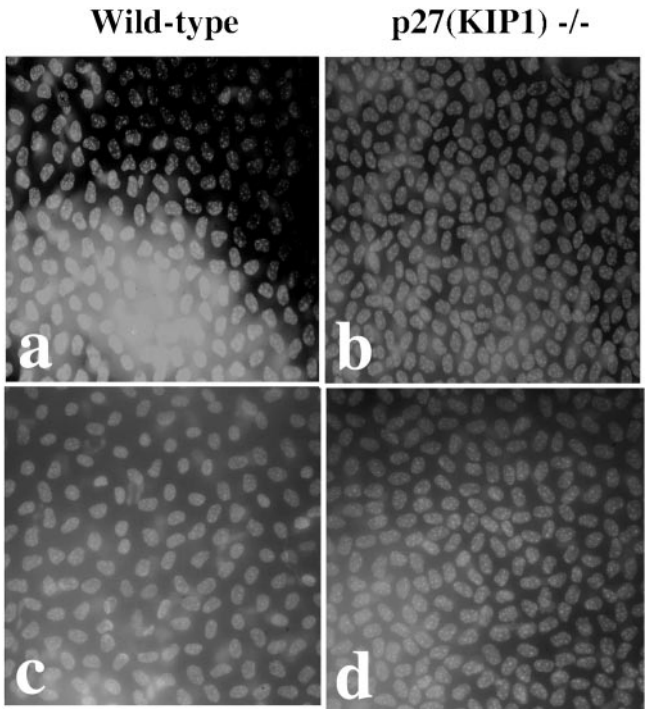


**FIGURE 3.** Nuclear staining with DAPI in wholemounts of endothelium from the center (**a**, **b**) and periphery (**c**, **d**) of the corneas of wild-type (**a**, **c**) and p27$^{KIP1-/-}$ (**b**, **d**) mice at 12 weeks of age. In the peripheral region, the peripheral margin was 310 $\mu$m from the iris root. Magnification, ×400.

More nuclei were seen in the endothelium of p27$^{KIP1-/-}$ mice than in that of wild-type mice.

The number of endothelial cells was then calculated within microscopic fields of identical size (345.6 × 276.5 $\mu$m) from the center and peripheral regions of the cornea. The number of endothelial nuclei in p27$^{KIP1-/-}$ mice was significantly higher than that in wild-type mice ($P < 0.0001$; $n = 7$; Fig. 4) in both the center and peripheral regions of the cornea. The number of endothelial nuclei was not significantly different between the center and peripheral regions of the cornea in either wild-type or p27$^{KIP1-/-}$ mice.

To evaluate the proliferative activity of the corneal cells, we examined the incorporation of BrdU, which was incorporated only into cells that were in the S phase.[23] When wild-type mice and littermate control animals were injected with BrdU between P7 and P10 and the eyes were analyzed at P11, BrdU-positive cells were detected among the epithelial cells (Fig. 5c, arrow) but they were not detected in corneal endothelial cells (Fig. 5c, arrowhead). In p27$^{KIP1-/-}$ mice, BrdU-positive cells were detected in the corneal endothelium (Fig. 5d, arrowhead) and the epithelium (Fig. 5d, arrow).

Finally, expression of PCNA was examined in the corneas of the p27$^{KIP1-/-}$ mice at 12 weeks after birth (Fig. 6). PCNA immunoreactivity was completely absent in the endothelium of the p27$^{KIP1-/-}$ mice at 12 weeks after birth.

## DISCUSSION

The p27$^{KIP1}$ protein prevents a cell in the G phase of the cell cycle from entering the S phase[6–9] PCNA is an essential replication factor[26] that is induced in the late $G_1$ phase and reaches a peak level in the S phase of the cell cycle.[19,20] In this study, p27$^{KIP1}$ was expressed in a small number of corneal endothelial cells, whereas many endothelial cells expressed PCNA on P1. In contrast, many endothelial cells in the central and peripheral cornea showed nuclear immunoreactivity for p27$^{KIP1}$ at P11 and 12 weeks after birth. PCNA-positive cells in the endothelium were rare on P11 and were completely absent at 12 weeks after birth. These results suggest that expression of p27$^{KIP1}$ in the developing corneal endothelium is well correlated with the cessation of the proliferation in wild-type mice.

To examine the role of p27$^{KIP1}$ in the proliferation of the corneal endothelium, DAPI was used for nuclear staining in wholemounts of corneal endothelium in the wild-type and the p27$^{KIP1-/-}$ mice at 12 weeks of age. The number of endothelial nuclei of the p27$^{KIP1-/-}$ mice was significantly higher than

Downloaded from iovs.arvojournals.org on 01/25/2021

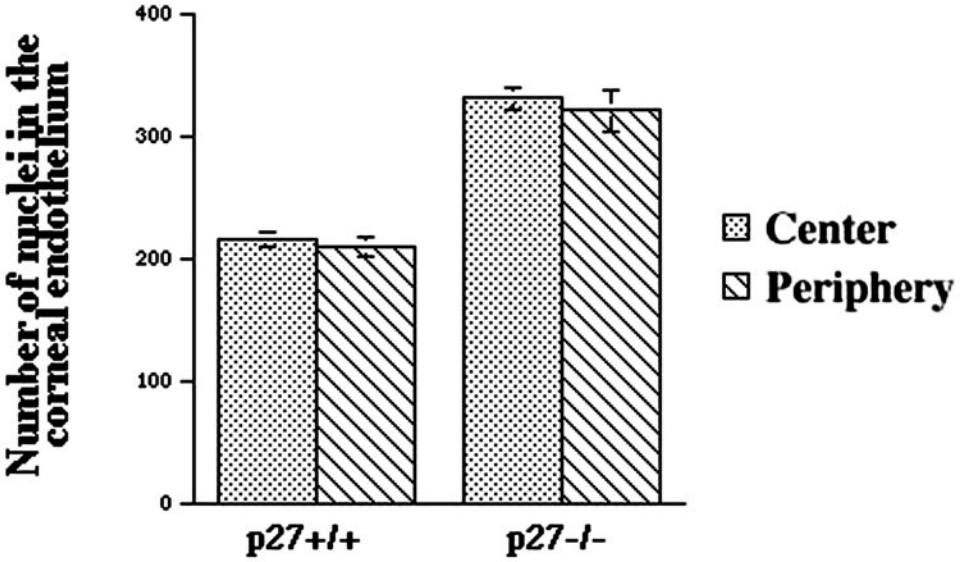


**FIGURE 4.** The number of nuclei in the corneal endothelium in wild-type and $p27^{KIP1-/-}$ mice at 12 weeks of age. Microscopic fields of identical size (345.6 × 276.5 μm) were chosen at the center and periphery of the cornea. For the peripheral region, the same-sized microscopic field was chosen at a position so that the peripheral margin was 310 μm from the iris root. Seven animals each were used for wild-type and $p27^{KIP1-/-}$ mice. $P < 0.0001$ for both center and peripheral retina.

that of wild-type mice. When mice were injected with BrdU between P7 and P10, and analyzed at P11, BrdU-positive cells were detected among the endothelial cells of the corneas in $p27^{KIP1-/-}$ mice, but they were not detected among those of wild-type mice. These results suggest that the period of proliferation of the corneal endothelium is prolonged in the $p27^{KIP1-/-}$ mice.

The number of endothelial nuclei was not significantly different between the center and peripheral region of the cornea in either wild-type or $p27^{KIP1-/-}$ mice. These results are in contrast to those in reports about the human cornea showing that the peripheral density of nuclei is higher than the central density.[27,28]

The specific mechanisms that inhibit proliferation on maturation of the corneal endothelium in vivo are unknown, but may be similar to the mechanisms that inhibit the proliferation of confluent cells. It has been reported that N-cadherin–mediated signaling is involved in contact inhibition of growth by inducing elevation of $p27^{KIP1}$ levels.[29] Together with recent reports showing that N-cadherin is expressed in the developing corneal endothelium,[30,31] these findings suggest that N-cadherin–mediated signaling may be involved in the inhibition of growth of corneal endothelial cells through the elevation of $p27^{KIP1}$ expression.

Recently, it was reported that $p27^{KIP1}$ is specifically recognized by Skp2 and that this step is a rate-limiting component of the machinery that ubiquitinates and degrades $p27^{KIP1}$.[16] An increase in $p27^{KIP1}$ expression has also been shown to be achieved by translational upregulation of $p27^{KIP1}$ synthesis[33] or by an increase in the amount of p27 polyribosome-bound mRNA.[34] Identification of the mechanism that regulates the protein level of the $p27^{KIP1}$ should be valuable in delineating the molecular pathways regulating the proliferation of corneal endothelial cells.

PCNA immunoreactivity in the endothelia of $p27^{KIP1-/-}$ mice was completely absent at 12 weeks after birth. This result suggested that proliferation of the endothelial cells in $p27^{KIP1-/-}$ mice stops until 12 weeks after birth and also that

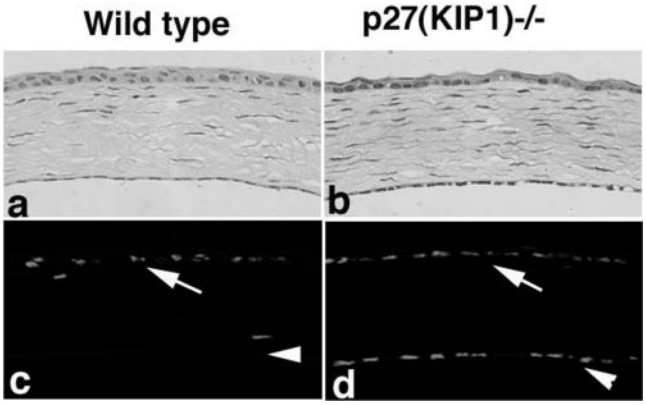


**FIGURE 5.** Hematoxylin staining (**a**, **b**) and immunodetection with BrdU (**c**, **d**) in the central cornea of wild-type (**a**, **c**) and $p27^{KIP1-/-}$ (**b**, **d**) mice. The mice were injected with BrdU between P7 and P10 and analyzed at P11. BrdU-positive cells were distributed among epithelial cells (**c**, *arrow*), but not among endothelial cells (**c**, *arrowhead*) of the central corneas in wild-type mice. In $p27^{KIP1-/-}$ mice, corneal endothelial cells (**d**, *arrowhead*) and epithelial cells (**d**, *arrow*) showed nuclear immunoreactivity for BrdU. Seven eyes each from wild-type and $p27^{KIP1-/-}$ mice were examined. Magnification, ×400.

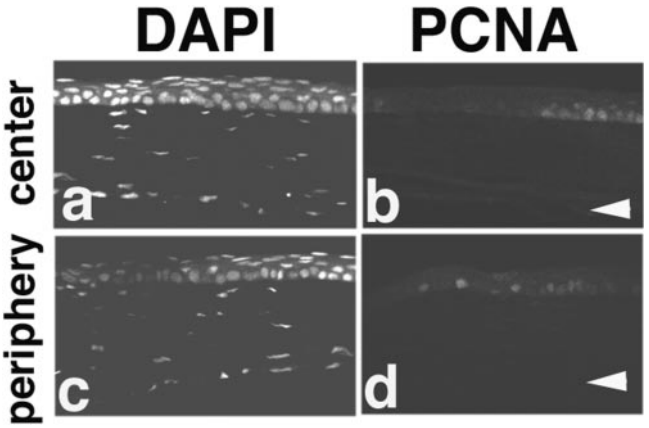


**FIGURE 6.** Nuclear staining with DAPI (**a**, **c**) and expression of PCNA (**b**, **d**) in the central (**a**, **b**) and peripheral (**c**, **d**) corneas of the $p27^{KIP1-/-}$ mice on 12 weeks after birth. Immunoreactivity for $p27^{KIP1}$ in the endothelium was completely absent at 12 weeks after birth (**b**, **d**, *arrowhead*). Magnification, ×400.

Downloaded from iovs.arvojournals.org on 01/25/2021

there must be other mechanisms that eliminate the excess endothelial cells.

### *Acknowledgments*

The authors thank Takae Ohyama for skillful technical assistance.

### *References*

1. Hay ED. Development of the vertebrate cornea. *Int Rev Cytol*. 1979;63:263–322.
2. Sherr CJ, Roberts JM. Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes Dev*. 1995;9:1149–1163.
3. Toyoshima H, Hunter T. p27, a novel inhibitor of G1 cyclin-Cdk protein kinase activity, is related to p21. *Cell*. 1994;78:67–74.
4. Kato JY, Matsuoka M, Polyak K, Massague J, Sherr CJ. Cyclic AMP-induced G1 phase arrest mediated by an inhibitor (p27Kip1) of cyclin-dependent kinase 4 activation. *Cell*. 1994;79:487–496.
5. Zhang P, Liegeois NJ, Wong C, et al. Altered cell differentiation and proliferation in mice lacking p57KIP2 indicates a role in Beckwith-Wiedemann syndrome. *Nature*. 1997;387:151–158.
6. Nourse J, Firpo E, Flanagan WM, et al. Interleukin-2-mediated elimination of the p27Kip1 cyclin-dependent kinase inhibitor prevented by rapamycin. *Nature*. 1994;372:570–573.
7. Reynisdottir I, Polyak K, Iavarone A, Massague J. Kip/Cip and Ink4 Cdk inhibitors cooperate to induce cell cycle arrest in response to TGF-beta. *Genes Dev*. 1995;9:1831–1845.
8. Coats S, Flanagan WM, Nourse J, Roberts JM. Requirement of p27Kip1 for restriction point control of the fibroblast cell cycle. *Science*. 1996;272:877–880.
9. Hirai A, Nakamura S, Noguchi Y, et al. Geranylgeranylated rho small GTPase(s) are essential for the degradation of p27Kip1 and facilitate the progression from G1 to S phase in growth-stimulated rat FRTL-5 cells. *J Biol Chem*. 1997;272:13–16.
10. Polyak K, Lee MH, Erdjument-Bromage H, et al. Cloning of p27Kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. *Cell*. 1994;78:59–66.
11. Pagano M, Tam SW, Theodoras AM, et al. Role of the ubiquitin-proteasome pathway in regulating abundance of the cyclin-dependent kinase inhibitor p27. *Science*. 1995;269:682–685.
12. Blagosklonny MV, Wu GS, Omura S, el-Deiry WS. Proteasome-dependent regulation of p21WAF1/CIP1 expression. *Biochem Biophys Res Commun*. 1996;227:564–569.
13. Thanos D, Maniatis T. NF-kappa B: a lesson in family values. *Cell*. 1995;80:529–532.
14. Yoshida K, Hu Y, Karin M. IkappaB Kinase alpha is essential for development of the mammalian cornea and conjunctiva. *Invest Ophthalmol Vis Sci*. 2000;41:3665–3669.
15. Zhang H, Kobayashi R, Galaktionov K, Beach D. p19Skp1 and p45Skp2 are essential elements of the cyclin A-CDK2 S phase kinase. *Cell*. 1995;82:915–925.
16. Carrano AC, Eytan E, Hershko A, Pagano M. SKP2 is required for ubiquitin-mediated degradation of the CDK inhibitor p27. *Nat Cell Biol*. 1999;1:193–199.
17. Tsvetkov LM, Yeh KH, Lee SJ, Sun H, Zhang H. p27(Kip1) ubiquitination and degradation is regulated by the SCF(Skp2) complex through phosphorylated Thr187 in p27. *Curr Biol*. 1999;9:661–664.
18. Yoshida K, Nakayama K, Nagahama H, et al. Involvement of p27(KIP1) degradation by Skp2 in the regulation of proliferation in response to wounding of corneal epithelium. *Invest Ophthalmol Vis Sci*. 2002;43:364–370.
19. Bravo R, Macdonald-Bravo H. Existence of two populations of cyclin/proliferating cell nuclear antigen during the cell cycle: association with DNA replication sites. *J Cell Biol*. 1987;105:1549–1554.
20. Kurki P, Vanderlaan M, Dolbeare F, Gray J, Tan EM. Expression of proliferating cell nuclear antigen (PCNA)/cyclin during the cell cycle. *Exp Cell Res*. 1986;166:209–219.
21. Nakayama K, Ishida N, Shirane M, et al. Mice lacking p27(Kip1) display increased body size, multiple organ hyperplasia, retinal dysplasia, and pituitary tumors. *Cell*. 1996;85:707–720.
22. Nakayama K, Nagahama H, Minamishima YA, et al. Targeted disruption of Skp2 results in accumulation of cyclin E and p27(Kip1), polyploidy and centrosome overduplication. *EMBO J*. 2000;19:2069–2081.
23. Yoshiki A, Hanazono M, Oda S, Wakasugi N, Sakakura T, Kusakabe M. Developmental analysis of the eye lens obsolescence (Elo) gene in the mouse: cell proliferation and Elo gene expression in the aggregation chimera. *Development*. 1991;113:1293–1304.
24. Yoshida K, Behrens A, Le-Niculescu H, et al. Amino-terminal phosphorylation of c-Jun regulates apoptosis in the retinal ganglion cells by optic nerve transection. *Invest Ophthalmol Vis Sci*. 2002;43:1631–1635.
25. Yoshida K, Kim JI, Imaki J, et al. Proliferation in the posterior region of the lens of c-maf−/− mice. *Curr Eye Res*. 2001;23:116–119.
26. Thacker SA, Bonnette PC, Duronio RJ. The contribution of E2F-regulated transcription to Drosophila PCNA gene function. *Curr Biol*. 2003;13:53–58.
27. Schimmelpfennig BH. Direct and indirect determination of non-uniform cell density distribution in human corneal endothelium. *Invest Ophthalmol Vis Sci*. 1984;25:223–229.
28. Amann J, Holley GP, Lee SB, Edelhauser HF. Increased endothelial cell density in the paracentral and peripheral regions of the human cornea. *Am J Ophthalmol*. 2003;135:584–590.
29. Levenberg S, Yarden A, Kam Z, Geiger B. p27 is involved in N-cadherin-mediated contact inhibition of cell growth and S-phase entry. *Oncogene*. 1999;18:869–876.
30. Reneker LW, Silversides DW, Xu L, Overbeek PA. Formation of corneal endothelium is essential for anterior segment development: a transgenic mouse model of anterior segment dysgenesis. *Development*. 2000;127:533–542.
31. Xu L, Overbeek PA, Reneker LW. Systematic analysis of E-, N- and P-cadherin expression in mouse eye development. *Exp Eye Res*. 2002;74:753–760.
32. Shirane M, Harumiya Y, Ishida N, et al. Down-regulation of p27(Kip1) by two mechanisms, ubiquitin-mediated degradation and proteolytic processing. *J Biol Chem*. 1999;274:13886–13893.
33. Hengst L, Reed SI. Translational control of p27Kip1 accumulation during the cell cycle. *Science*. 1996;271:1861–1864.
34. Millard SS YJ, Nguyen H, Pagano M, Kiyokawa H, Koff A. Enhanced ribosomal association of p27(Kip1) mRNA is a mechanism contributing to accumulation during growth arrest. *J Biol Chem*. 1997;14:7093–7098.

Downloaded from iovs.arvojournals.org on 01/25/2021