Cis-regulatory elements of the mouse *Krt1.12* gene

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Key words: Keratin 12; gene gun; footprinting; corneal epithelium; electromobility shift assay.

INTRODUCTION

Keratins are a group of water-insoluble proteins that form the 10 nanometer (nm) intermediate filaments found in epithelial cells.21 The major function of the cytoskeletal network formed by keratins is to provide a rigid epithelial cell layer that protects underlying tissues from the environment.11 The proposition is substantiated by mutations of K1/K10 and K5/K14, which manifests epidermolysis hyperkeratosis and epidermolysis bullosa simplex in skin, respectively. Whereas mutations of the K12 gene in human and ablation of the K12 gene via gene targeting in mice results in fragile corneal epithelium, a clinical manifestation characteristic of Meesmann's corneal dystrophy.14,15

The approximately thirty different keratin proteins are subdivided into acidic type I and basic type II 13,21,25,30. *In vivo*, a basic keratin usually heterodimerizes or "pairs" with a particular acidic keratin.8,13,21,25,27,28,30,33,37. The expression of keratin pairs is tissue-specific, differentiation regulated, and development-dependent. For example, K5/K14 keratin pair is found in the basal cell layer of all stratified epithelium; whereas, K1/K10 keratin pair is expressed by suprabasal and superficial epidermal epithelial cells. The K3/K12 pair is regarded as a marker for cornea-type epithelial differentiation. It should be noted however that the expression of K3 is not limited to cornea epithelium; it has been detected in several other tissues.5,7,8,36 We have cloned the mouse cornea-specific K12 gene and demonstrated that the expression of K12 is restricted to the corneal epithelium

5,6,18,22,39,40

Many keratin genes are regulated at the transcriptional level in a coordinate manner during keratinocyte differentiation. 9,10 Tissue-specific keratin expression is regulated by a complex collaboration between ubiquitous and tissue-specific transcription factors and is dependent on the turn-on and -off of a variety of regulatory genes. 11,26,31 There are several transcriptional factors known for the regulation of keratin gene expression. For example, AP-2 and SP-1 have been shown to be able to regulate the expression of K1, K3, K5, K6, and K14. 3,16,17,38 Furthermore, the regulation of a tissue-specific keratin gene always needs coordinated expression of several transcription factors. 3,16,17,38

Recently, we successfully used Gene Gun, a particle-mediated gene transfer technique, to deliver K12-promoter reporter genes to rabbit corneal epithelial cells *in vivo* and identified a 0.6 kb DNA fragment 5' flanking *Krt1.12* possibly contained corneal epithelial cell-specific regulatory *cis*-DNA elements. 29 In the present study, we further examined the 0.6 kb 5' flanking region in the K12 gene to determine the *cis*-regulatory elements accounting for corneal epithelial cell-specific expression. To do this, we used both *in vitro,* DNase I footprinting and electrophoretic mobility shift assays (EMSA), and *in vivo*, Gene Gun, techniques to elucidate the epithelial cell-specific *cis*-regulatory element(s) of *Krt1.12*.

MATERIALS AND METHODS

Plasmids

Two plasmid DNA constructs, pCMVβ and pNASSβ (Clontech, Palo Alto, CA), were used as positive and negative control reporter genes. Three reporter gene constructs, 0.2 KZ, 0.4 KZ, and 0.6 KZ were prepared by cloning 127 bp, 327 bp, and 527 bp sequence plus 40 bp of exon 1 untranslated region of the *Krt1.12* gene to pNASSβ plasmid that contained a *lac* Z reporter gene.

In vivo Particle-Mediated Gene Transfer: Gene Gun

All animal experiments were performed according to the ARVO resolution on the use of animals in vision research. Plasmid DNA purified using Qiagen® columns (Qiagen, Chatsworth, CA) was coated onto 0.6 μm, 1.0 μm or 1.6 μm gold particles (5 μg DNA per mg of gold), loaded into Tefzel® tubing (Biorad, Hercules, CA), and transiently transfected into New Zealand white rabbit (~2 kg) corneal epithelium using the Helios™ Gene Gun System (Biorad) at 200 psi according to Shiraishi et al. 11 Samples were collected 48 h after delivery and subjected to β-galactosidase activity analysis.

Preparation of enzyme extracts

Excised tissue specimens were minced with a razor blade, and 0.5 ml of extraction buffer (0.25 M Tris-HCl, pH 7.4, 0.1 % Tween 20) was added. The samples were subjected to 3 freeze-thaw cycles, 5 min on dry ice and 5 min at 37°C. The supernatants

were collected by centrifugation at 13,000 x g at 4°C for 10 min.

-Galactosidase Activity Assay And Whole Mount -galactosidase Histochemical Staining

Aliquots of supernatant were incubated in a 0.3 ml mixture containing 50 mM 2-mercaptoethanol, 1 mM $MgCl_2$, 1.33 mg/ml o-nitrophenyl β-galactopyranoside and 0.1 M phosphate buffer, pH 7.0 at 37°C for 1 to 5 h. A 0.7 ml aliquot of 1 M Na_2CO_3 was added to terminate the reaction. The enzyme activity was determined by comparing the optical density at 460 nm to that of purified β-galactosidase (Boehringer and Mannheim, Indianapolis, IN). The promoter activity of KZ reporter gene constructs was calculated as a fold-increase of the enzyme activities derived from the promoter-less pNASSβ construct.

For whole mount X-gal staining, the eyeball was enucleated, fixed immediately with 4% paraformaldehyde in PBS at 4°C for 2 h, and washed with PBS buffer (pH 7.0) containing 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, and 1.4 mM KH_2PO_4. Staining was carried out at 30°C for 16 h in a solution of 5-bromo-4-chloro-3-indolyl-β-galactopyronoside (X-gal, Sigma) at a final concentration of 0.4 mg/ml. Following staining, eyeballs were rinsed with PBS and photographed as whole mounts as previously described. 29.

Preparation of Nuclear Extracts

Nuclear extracts were prepared as described by Andrews and Faller.1 Briefly, bovine corneal epithelium was scraped from enucleated eyes into ice-cold PBS, and then homogenized by Teflon-glass in homogenization buffer (2 ml/gm tissue) containing 0.1% Triton X-100, 10 mM HEPES (pH 7.6), 25 mM KCl, 1 mM EDTA, 2 M sucrose, 0.5 mM spermidine, 0.15 mM spermine, 10% (v/v) glycerol in DEPC H_2O. The homogenate was then 3-fold diluted in the same buffer without glycerol and centrifuged at 104,000 x g for 30 min on a 10 ml cushion of homogenization buffer. Pellets were then combined and resuspended in 15-20 ml of a 9:1 (v/v) mixture of homogenization buffer and glycerol, using a Teflon-glass homogenizer. This homogenate was layered on a 10 ml cushion of the 9:1 mixture and centrifuged as above. The nuclei were homogenized in cold lysis buffer containing 10 mM HEPES (pH 7.6), 0.1 M KCl, 3 mM $MgCl_2$, 0.1 mM EDTA, 10% glycerol, 1 mM DTT, and 0.1 mM PMSF in DEPC H_20 with a glass-glass homogenizer. The absorbance was then checked with a spectrophotometer at a 260 nm wavelength and diluted in lysis buffer to 10 A260 unit/ml. The nuclear extract was subjected to two steps of ammonium sulfate fractionation by first incubating for 30 min in a 10% vol of 4 M $(NH_4)_2SO_4$ (pH 7.9), followed by centrifugation (100,000 x g for 60 min). The nuclear proteins in the supernatant were then precipitated by adding solid $(NH_4)_2SO_4$ (0.39 g/ml) and collected

by centrifugation (100,000 x g, 60 min). The nuclear protein extract was resuspended and dialyzed in buffer containing 25 mM, 10 mM HEPES, 40 mM KCl, 10% glycerol, 0.1 mM EDTA, 1 mM DTT, in DEPC H_2O. The extract was then aliquoted and stored at -70 °C.

DNase I Footprinting Analysis

Three DNA probes (-332 bp to –109 bp, -394 bp to –131 bp, and -599 bp to –375 bp,) were prepared via PCR (primers listed in Table 2) and 5' labeled with [γ-^{32}P] ATP using T4 polynucleotide kinase. A 50 µl mixture containing 10 mM Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 5 mM $CaCl_2$, 50 mM KCl, 500 nM dithiothreitol, 0.05 mg/ml bovine serum albumin, and 40.5 ng/µl poly-dIdC was incubated with crude nuclear extracts (75 µg) on ice for 15 minutes. The mixture was then incubated for an additional 45 minutes with 100,000 CPM of DNA probe. Variable amounts of DNase I were added to the mixture and incubated at 25°C for 1 min 30 sec. This reaction was stopped with 50 µl of stop buffer (0.2 M NaCl, 30 mM EDTA, 1% SDS, and 0.1 mg/ml yeast tRNA). Digested DNA probes were purified by phenol extraction and ethanol precipitation and separated on 6.5% denaturing polyacrylamide gels. Dried gels were exposed to Kodak XAR film with intensifying screens for 24 hours at –80^{o}C. G and G+A sequencing reactions were performed to determine the positions of the protected regions. Negative control reactions were performed in the absence of nuclear protein extracts. Regions

protected by nuclear proteins were numbered according to the nucleotide positions relative to the transcription start site of the *Krt1.12* gene.

Electrophoretic Mobility Shift Assays

Gel-shift analysis of the potential tissue specific region of the *Krt1.12* promoter was performed with ^{32}P labeled double-stranded synthetic oligonucleotide probes (-182 bp to –111 bp and -256 bp to –193 bp) (Table 3), and crude nuclear extracts prepared from bovine corneal epithelial cells as previously described. Twenty microliter binding reactions were performed, which contained 10 µg of nuclear proteins, 20,000 cpm double strand oligonucleotides end-labeled with [γ-^{32}P] ATP, 12 mM HEPES (pH 7.9), 10% glycerol, 4 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.3 mg/ml bovine serum albumin, 1 mM dithiothreitol (DTT), 0.1 mg/ml poly-dAdT, and 60 mM KCl. Each labeled probe was competed with an excess of the same unlabeled double-stranded synthetic oligomer. Binding mixtures were separated on a nondenaturing 5% polyacrylamide gel and exposed to Kodak XAR film.

Immunohistochemistry

Paraffin sections of mouse eyes were deparaffinized and incubated in PBS for 30 min. The tissue section was then blocked with BSA and 3% nonfat milk in PBS for 60 min at room temperature. Rabbit antibodies against mouse c-jun/AP-1 and c/EBPβ (Santa Cruz Biotech. Inc., Santa Cruz, CA) were diluted 25x in the blocking solution

and incubated on the tissue sections at 4°C overnight. The sections were then washed in PBS. A goat anti-rabbit Alexa Fluor 488 (Molecular Probes Inc. Eugene, OR) secondary antibody was used for immunoreactivity detection using fluorescence microscopy.

RESULTS

Analysis of promoter activities of the 5'-Flanking Region of the *Krt1.12* Gene

It was previously demonstrated that the –0.6 kb 5'flanking DNA fragment could direct cornea-specific expression of a β-galactosidase gene *in vivo* 29. In an attempt to identify the region where cornea-specific 5' flanking *cis*-regulatory elements exist in the *Krt1.12* gene we tested the 0.2 KZ, 0.4 KZ and 0.6 KZ constructs *in vivo.* Rabbit corneas, conjunctivas, and skin were transiently transfected with the 0.2, 0.4, and 0.6 KZ constructs using gene gun. Figure 1 shows that all three promoter constructs express 7-10 fold β-gal activities above the promoter-less pNASSβ construct in cornea. In skin and conjunctiva, the 0.4 and 0.6 KZ constructs yield insignificant β-galactosidase activities in comparison to those of pNASSβ; whereas, the 0.2 KZ construct produces 3-fold β-galactosidase activity above pNASSβ. The observations suggest that the 0.2 KZ construct does not have cornea-specific *cis*-regulatory elements for the expression of the β-galactosidase reporter gene. And, the 0.4 KZ and 0.6 KZ constructs did show cornea-specific β-galactosidase activities when compared to that of

conjunctiva and skin. To further elucidate this suggestion whole mount X-gal staining was performed with transfected rabbit eyes. Figure 2 demonstrates that the 0.2 KZ construct (Panel A) shows β-gal expression in both the cornea and conjunctiva. The 0.4 KZ (Panel B) and 0.6 KZ construct (Panel C) show reporter gene expression exclusively in the cornea. The observation suggested that a cornea-specific regulatory element(s) might exist in the –327 to +40 bp region of *Krt1.12* gene.

DNase I Footprinting Assay

Since the *in vivo* data indicates that the 0.4 KZ reporter gene construct has cornea-specific promoter activity, DNase I footprinting was performed to further identify the DNA sequences that may account for cornea-specific *Krt1.12* gene expression. Two footprinting probes (-394 bp to –131 bp, and –331 bp to –109 bp) were used to identify the possible transcription factor binding site(s) that the previously described data suggested exist in this region. Bovine epithelial nuclear extract protection was seen for a 72 bp fragment in the –182 bp to –110 bp region of the *Krt1.12* gene. Also, protection existed in 2 other regions of the *Krt1.12* gene, from –231 bp to –193 bp and from –256 to –243 (Fig3).

Electrophoretic Mobility Shift Assays (EMSA)

EMSAs were performed to further identify the 5' flanking region of *Krt1.12*, which may bind to nuclear factors. Synthetic oligonucleotides used in EMSAs (Table 3) were

designed from the regions protected by epithelial nuclear proteins in the footprinting assays. EMSA of site from –182 to -111 and –256 to –193 showed a binding complex with nuclear proteins from corneal epithelial cells, but the complex could also be detected at lower affinity with nuclear proteins of retinal cells (Fig. 4) The radioactive labeled complex was competed and eliminated by a 400- or 800-fold molar excess of the unlabeled oligonucleotides termed self-competitor. Sequence analysis suggests that several transcriptional factors can potentially bind to this region of the of *Krt1.12* promoter, which includes c-jun/AP-1 and c/EBPβ binding sites (Fig. 5).

Immunohistochemistry

Consensus sequence analysis revealed potential c-jun/AP-1 and c/EBPβ (Fig 5) binding sites in the 5' flanking *Krt1.12* region. Immunohistochemistry was performed to determine if the expression of these two transcription factors may be present in corneal epithelial cells, but absent in limbal basal epithelial cells and can be correlated with that of keratin 12 expression. Figure 6 demonstrates the presence of c-jun/AP-1 and c/EBPβ in the nucleus of all mouse corneal epithelial cell layers. However, c/EBPβ appears to have a significantly lower expression level in the limbal region of the cornea as compared to c-jun/AP-1. The observation is consistent with the expression of keratin 12 in corneal epithelial cells, since keratin 12 is only expressed in corneal epithelial cells, but not in the basal layers of the limbus.

Discussion

Although many keratin genes have been cloned, the 5'-upstream sequences of only a few of them have been found to function as tissue-specific promoters by *in vitro* analysis of cultured keratinocytes and *in vivo* in transgenic mice. In the present study, we discovered the 0.4 kb 5' flanking region of the mouse *Krt1.12* gene appeared to contain the *cis*-regulatory elements necessary for cornea-specific expression as demonstrated by *in vivo* transfection of β-galactosidase reporter genes with Gene Gun. Analyses using foot printing and EMSA further identifies two regions –256 bp to –193 bp and –182 bp to –111 bp 5' to the transcription initiation site that may play a role in Krt1.12 gene expression by corneal epithelial cells.

Analysis of sequence comparison to the Gene Bank (NCBI GenBank database, Blast web client software) revealed several possible transcription factor binding sites including, AP-1 (-240 to –250 bp and –124 bp to –133 bp) and c/EBPβ (-133 bp to –147 bp, -229 to –242 bp, and –226 to –243 bp) and many others in the sense and antisense orientation as shown in Figure 5.

AP-1 (activation protein 1) was well known to be a regulator of many keratin genes including K1, K3, K5, K8, K10, and K18, which are expressed by basal and suprabasal epithelial cells of stratified epithelium, as well as single cell-layer epithelium. [4,19,23,34,35,38] Immunohistochemistry detected AP-1 in all cell layers

of the cornea which is consistent with the expression patterns of keratinocyte differentiation. The retina was also positive for AP-1 (data not shown) which in parts explains the EMSA data.

Expression of K12 is limbal basal negative until after migration from the limbal basal layer. Our c/EBPβ transcription factor immunostaining shown in Fig. 6 was suprabasal positive in the nuclei of corneal epithelial cells, but expression appears down regulated in the limbal basal region especially with respect to c/EBPβ. C/EBP family members (α, β, γ, and δ) contain the bZIP region. The bZIP region is characterized by two motifs, one of which is involved in DNA binding, and the other a leucine zipper involved in dimerization. c/EBPβ can homodimerize or heterodimerizes with other family members and other transcription factors (NFκB, p65, p50, and rel family members) {Stein, Cogswell, et al. 1993 18 /id}. The interaction of c/EBPβ is believed to be a gene regulation mechanism involving an interaction of various transcription factors.

In essence this study identifies the 5' flanking region of the *Krt1.12* gene responsible for tissue-specific expression, provides evidence of transcription factor cooperation for tissue-specific gene expression, and potentially elucidates an *in vivo* transfection technique for controlled gene delivery and expression. This study further indicates the regulation of *Krt1.12* gene expression may result from a coordinated

function of several transcriptional factors including AP-1/c-jun and c/EBPβ.9,10.

Table 1. Primers Used for DNase I Footprinting

Probe Length (bp)	Sense Primer	Antisense Primer
-599~-375	ACTTCCAAACA GCTGGCTC	CGGTTCAGTATAAG CCAACC
-394~-131	GGTTGGCTTATA CTGAACCG	TGCATCGTGAAAAT TCACTG
-331~-109	GGCTTCGGGAA GGATCCTT	CCAGCTCAGCAGGT ATGTGA

Table 2. Probes for Electrophoretic Motility Shift Assays

Probe Length (bp)	Sequence
-256~-193	ACTGAAGGTGACAGATTCCTTACGACAGCCTA
	TCTGCTCCACCCAGCCTTCTTTCTTGTGTGTC
-182~-111	TGGTAATGGTTATTCGATTATAGCTATATCAGTGAAT
	TTTCACGATGCATAAATCACATACCTGCTGAGCTG

Figure 1

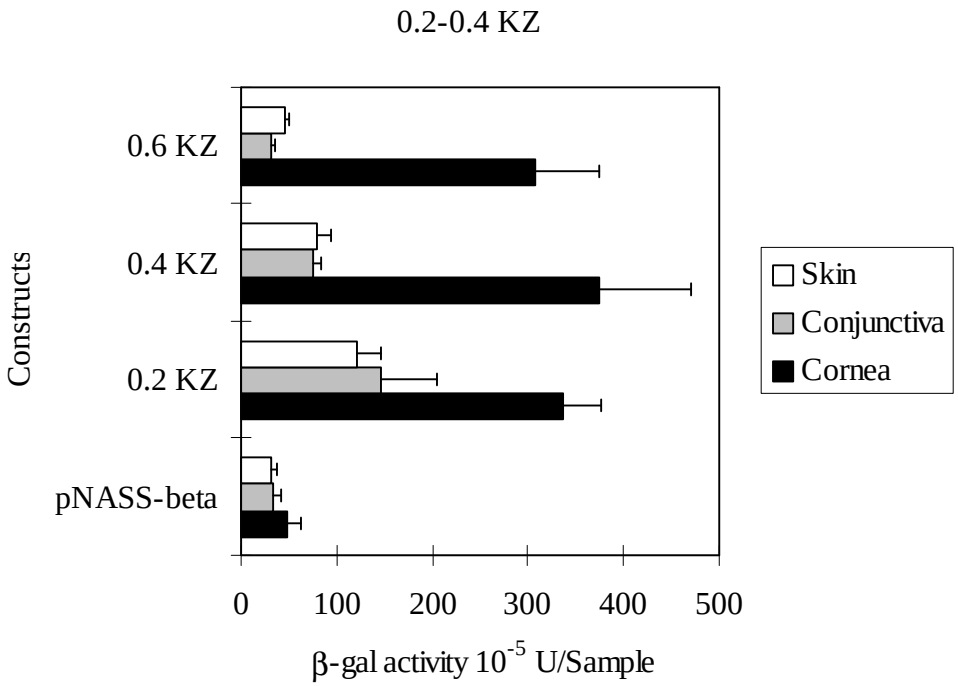
0.2-0.4 KZ
0.6 KZ
0.4 KZ
0.2 KZ
pNASS-beta
Constructs
Skin
Conjunctiva
Cornea
0
100
200
300
400
500
β-gal activity 10-5 U/Sample

Figure Legend

Figure 1. In vivo β-gal expression by *Krt1.12* promoter-β-gal constructs in rabbit corneas, conjunctivas and skin. 0.6 μm gold particles coated with *Krt1.12* constructs were bombarded onto rabbit corneas at 150 psi, and corneal tissue were collected 48 h after delivery. 1.6 μm gold particles coated with *Krt1.12* constructs were bombarded onto rabbit bulbar conjunctivas at 150 psi, and conjunctival tissue were collected 48 h after delivery. 1.6 μm gold particles coated with *Krt1.12* constructs were bombarded onto rabbit dorsal skin at 400 psi (8 mm in diameter), and skin tissue were collected 48 h after delivery. Values are averaged of β-gal activities ± standard deviations per specimen.

Figure 2. *In situ* histochemical staining of β-gal in cornea and conjunctiva. Keratin 12 promoter-β-gal constructs were coated onto 0.6 μm gold particles and delivered to cornea/conjunctival junctions at 200 psi. The tissues were collected at 48 h and subjected to staining with X-gal as described in METHODS. pNASSβ generates very few positive reactions in both cornea and conjunctiva. The 0.2 kb generates positive reactions in both cornea and conjunctiva. The expression of β-gal by the 0.4 kb constructs of the 5'flanking sequence of *Krt1.12* gene is restricted to the cornea.

Figure 3. *In vitro* footprinting of *Krt1.12* promoter. DNA fragments representing

-394~-131 and -331~-109 were prepared from polymerase chain reactions after uniquely labeled at the 5-end of the sense primers, and were digested with DNase I as described in METHOD. The concentration of nuclease was 100 ng/ml and nuclear protein was 75 μg. Left lanes showed the control reaction without corneal nuclear protein.

Figure 4. Nuclear extracts from bovine corneal epithelial cells bind regions shown in footprinting assays. The synthetic double-stranded DNA probes corresponding to region of –111 bp to –182 bp and –193 bp to –256 bp were labeled with [γ-^{32}P]dCTP and used in EMSAs. Crude nuclear extracts were prepared from bovine corneal epithelial cells by direct scrapping and retinal tissue. In both regions, the bindings were specific and could be competed with unlabeled probe. However, the probes could also bind the nuclear extracts from retinal tissue.

Figure 5. Schematic of consensus sequence analysis of the sense and antisense regions (-111 to –182 bp and –193 to –256 bp) of the *Krt1.12* gene showing the transcription factor binding sites generated by computer analysis using the NCBI GenBank database and Blast web client software.

Figure 6. Immunohistochemical staining of mouse eye paraffin sections of central cornea or the limbal region probed with anti-c-jun/AP1, anti-c/EBPβ, and a

pre-immune control. Immunoreactivity was detected by immunofluorescence.

Reference List

1. N. C. Andrews and D. V. Faller (5-11-1991) Nucleic Acids Res. 19, 2499.

2. W. J. Boyle, T. Smeal, L. H. Defize, P. Angel, J. R. Woodgett, M. Karin, and T. Hunter (2-8-1991) Cell 64, 573.

3. C. Byrne and E. Fuchs (1993) Mol.Cell Biol. 13, 3176.

4. J. Casatorres, J. M. Navarro, M. Blessing, and J. L. Jorcano (8-12-1994) J.Biol.Chem. 269, 20489.

5. C. Chaloin-Dufau, T. T. Sun, and D. Dhouailly (12-1-1990) Cell Differ.Dev. 32, 97.

6. W. Y. Chen, M. M. Mui, W. W. Kao, C. Y. Liu, and S. C. Tseng (1994) Curr.Eye Res. 13, 765.

7. D. Cooper, A. Schermer, and T. T. Sun (1985) Lab Invest 52, 243.

8. D. Cooper and T. T. Sun (4-5-1986) J.Biol.Chem. 261, 4646.

9. R. L. Eckert, J. F. Crish, and N. A. Robinson (1997) Physiol Rev. 77, 397.

10. R. L. Eckert and J. F. Welter (1996) Mol.Biol.Rep. 23, 59.

11. E. Fuchs (1990) J.Cell Biol. 111, 2807.

12. H. Gille, A. D. Sharrocks, and P. E. Shaw (7-30-1992) Nature 358, 414.

13. S. M. Hollenberg, R. Sternglanz, P. F. Cheng, and H. Weintraub (1995) Mol.Cell Biol. 15, 3813.

14. A. D. Irvine, L. D. Corden, O. Swensson, B. Swensson, J. E. Moore, D. G. Frazer, F. J. Smith, R. G. Knowlton, E. Christophers, R. Rochels, J. Uitto, and W. H. McLean (1997) Nat.Genet. 16, 184.

15. W. W. Kao, C. Y. Liu, R. L. Converse, A. Shiraishi, C. W. Kao, M. Ishizaki, T. Doetschman, and J. Duffy (1996) Invest Ophthalmol.Vis.Sci. 37, 2572.

16. A. Leask, C. Byrne, and E. Fuchs (9-15-1991) Proc.Natl.Acad.Sci.U.S.A 88, 7948.

17. A. Leask, M. Rosenberg, R. Vassar, and E. Fuchs (1990) Genes Dev. 4, 1985.

18. C. Y. Liu, G. Zhu, R. Converse, C. W. Kao, H. Nakamura, S. C. Tseng, M. M. Mui, J. Seyer, M. J. Justice, M. E. Stech, and . (10-7-1994) J.Biol.Chem. 269, 24627.

19. B. Lu, J. A. Rothnagel, M. A. Longley, S. Y. Tsai, and D. R. Roop (3-11-1994) J.Biol.Chem. 269, 7443.

20. R. Marais, J. Wynne, and R. Treisman (4-23-1993) Cell 73, 381.

21. R. Moll, W. W. Franke, D. L. Schiller, B. Geiger, and R. Krepler (1982) Cell 31, 11.

22. P. D. Moyer, A. H. Kaufman, Z. Zhang, C. W. Kao, A. G. Spaulding, and W. W. Kao (1996) Differentiation 60, 31.

23. R. G. Oshima, L. Abrams, and D. Kulesh (1990) Genes Dev. 4, 835.

24. B. J. Pulverer, J. M. Kyriakis, J. Avruch, E. Nikolakaki, and J. R. Woodgett (10-17-1991) Nature 353, 670.

25. R. A. Quinlan, D. L. Schiller, M. Hatzfeld, T. Achtstatter, R. Moll, J. L. Jorcano, T. M. Magin, and W. W. Franke (1985) Ann.N.Y.Acad.Sci. 455, 282.

26. R. H. Rice and H. Green (1977) Cell 11, 417.

27. A. Schermer, S. Galvin, and T. T. Sun (1986) J.Cell Biol. 103, 49.

28. A. Schermer, J. V. Jester, C. Hardy, D. Milano, and T. T. Sun (1989) Differentiation 42, 103.

29. A. Shiraishi, R. L. Converse, C. Y. Liu, F. Zhou, C. W. Kao, and W. W. Kao (1998) Invest Ophthalmol.Vis.Sci. 39, 2554.

30. P. M. Steinert and D. R. Roop (1988) Annu.Rev.Biochem. 57, 593.

31. A. Stoler, R. Kopan, M. Duvic, and E. Fuchs (1988) J.Cell Biol. 107, 427.

32. B. Su, E. Jacinto, M. Hibi, T. Kallunki, M. Karin, and Y. Ben Neriah (6-3-1994) Cell 77, 727.

33. T. T. Sun, S. C. Tseng, A. J. Huang, D. Cooper, A. Schermer, M. H. Lynch, R. Weiss, and R. Eichner

(1985) Ann.N.Y.Acad.Sci. 455, 307.

34. Y. Takemoto, Y. Fujimura, M. Matsumoto, Y. Tamai, T. Morita, A. Matsushiro, and M. Nozaki (5-25-1991) Nucleic Acids Res. 19, 2761.

35. Y. Tamai, Y. Takemoto, M. Matsumoto, T. Morita, A. Matsushiro, and M. Nozaki (8-15-1991) Gene 104, 169.

36. Z. G. Wei, R. L. Wu, R. M. Lavker, and T. T. Sun (1993) Invest Ophthalmol.Vis.Sci. 34, 1814.

37. R. A. Weiss, R. Eichner, and T. T. Sun (1984) J.Cell Biol. 98, 1397.

38. R. L. Wu, S. Galvin, S. K. Wu, C. Xu, M. Blumenberg, and T. T. Sun (1993) J.Cell Sci. 105 (Pt 2), 303.

39. R. L. Wu, G. Zhu, S. Galvin, C. Xu, T. Haseba, C. Chaloin-Dufau, D. Dhouailly, Z. G. Wei, R. M. Lavker, W. Y. Kao, and . (1994) Differentiation 55, 137.

40. G. Zhu, M. Ishizaki, T. Haseba, R. L. Wu, T. T. Sun, and W. W. Kao (1992) Curr.Eye Res. 11, 875.