Regulation of K12 Keratin Gene in Corneal Epithelial Cells

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INTRODUCTION

Keratins are a group of water-insoluble proteins that form 10 nm intermediate filaments in all epithelial cells. [1] About 30 different keratin molecules have been identified and can be divided into acidic and basic neutral subfamilies in tissue-specific manners.[1-4] *In vivo,* a basic keratin is usually co-expressed and "paired" with a particular acidic keratin. [1;3-5] According to their mode of expression and their association with progressively more complicated structures, the keratin pairs can be divided into several categories: (1) keratins of the simple epithelia include the K8/K18 pair and several small MW keratins; (2) keratins expressed by the basal cells of all stratified squamous epithelia, K5/K14 pair; (3) kertins expressed by the suprabasal cells of all stratified epithelia that become hyperplastic due to diseased or wound conditions or tissue culture stimuli, the K6/K16 pair; and (4)keratins expressed by suprabasal cells of normally differentiating stratified squamous epithelia.[6] For example, expression of the K12/K3 pair has been regarded as a marker for cornea-type epithelial differentiation.[5;7;8] The K12/K3 intermediate filaments are vital for corneal epithelial cell integrity, phsycal stability and rigidity. Mutations of K12 and K3 genes in human, and ablation of K12 gene via gene targeting in mice result in fragile corneal epithelium, a clinical manifestation characteristics of Meesmann's corneal dystrophy.{[9]}[10] Although K12/K3 pair is considered as the cornea-type differentiation marker, recent evidence indicates that K3 can be expressed in several tissues other than the corneal epithelium.[7]}[11]} In contrast, we have cloned the mouse cornea-specific K12 gene and

demonstrated that the expression of K12 is restricted to the corneal epithelium.[7;12-17]

The expression of tissue-specific keratin was dependent on the turn-on and/or turn-off of a variety of genes.[18-20] Many of these keratinocytes genes are regulated at the transcriptional levels in a coordinate manner during keratinocyte differentiation.[21;22]Most of these genes are also regulated in an independent manner by at least one regulatory agent and the gene regulatory regions do not appear to be conserved. [21] The fine-tuned regulation of certain keratin genes has been shown in pairwise (e.g., K1-K10, K3-K12, K5-K14, and K6-K16) and some of these pairs are expressed reciprocally (e.g., K3-K12 and K5-K14).[23-26]There are several transcriptional factors known for the regulation of keratin gene expression. For example, AP-2 and SP-1have been shown to be able to regulate the expression of K1, K3, K5, K6, K12, and K14.[6;27-29]Furthermore, the regulation of keratin gene expression always needs the combination of several transcription factors to express tissue specificity. [6;27-29] We have attempted, but failed to identify the cornea epithelial, K12, cell-specific promoter using conventional *in vitro* transfection of cultured corneal epithelial cells and *in vivo* transgenic mice.[30]But recently, we successfully used Gene Gun, particle-mediated gene transfer, to deliver K12 transgenes to corneal epithelial cells *in vivo*.[30] We observed that 2.5 kb DNA fragment 5' flanking *Krt1.12* possibly contained corneal epithelial cell-specific regulatory cis-DNA elements and PAX 6 is an important transcription factor for keratin 12 expression. In present study, more fine-tuned deletion

constructs with Gene Gun analysis as well as DNase I footprinting and electrophoretic motility shift assays (EMSA) were performed to elucidate the regulation of tissue specificity in 2.5 kb DNA fragment 5' flanking *Krt1.12*.

MATERIALS AND METHODS

Plasmids.

Two plasmid DNA constructs i.e., pCMVβ and pNASSβ (Clontech, Palo Alto, CA), were used as control reporter genes. A series of keratin 12 promoter fragments were prepared and cloned into pNASSβ vector as shown in Fig. 1. The 2.5 kb *Krt1.12* promoter construct was derived by *Nru/XhoI* cut from a 5.0 kb *Krt1.12* promoter construct and ligated to *Ehe/XhoI* cut pNASSβ. The 2.0 kb *Krt1.12* promoter was derived by *SacI/XhoI* cut from a 2.5 KB *Krt1.12* promoter and ligated into *EcoRI/XhoI* cut pNASSβ using an *Eco/Asc/Sac* adaptor. A 4.5 kb *EcoRI/HindIII* fragment from 0.6 KB containing the β-gal reporter driven by the 3' 600 bp of the K12 promoter was ligated to a 3.7 kb *EcoRI/HindIII* fragment from 2.0 kb *Krt1.12* constructs containing the vector backbone of pNASSβ and 5' 1000 bp of the K12 promoter. The resulting a 2 kb0.4 kb vector is identical to 2.0 kb with the exception that is missing 400 bp of the K12 promoter starting at the 975 bp *EcoRI* site and extending 400 bp 3' toward the 3' end of the promoter. The 2 kb construct were produced by the same method as the 2 kb 0.6 kb constructs. The 3' ends of these constructs were created by digestion with *XhoI* and corresponded to position +40 on the transcriptional initiation site. The fragments were cloned in the sense orientation about the β-galactosidase gene, and constructs were numbered according to the sequence positions of the 5'end. Other deletion constructs were produced by polymerase chain reaction

from 2.5 kb *Krt1.12* constructs, and all constructs were confirmed by DNA sequencing. All of these construct and primers used for polymerase chain reaction were shown in Table1.

***In vivo* Gene Transfer by Gene Gun.**

Plasmid DNA purified by Qiagen® columns (Qiagen, Chatsworth, CA) was coated onto gold particles of 0.6 μm, 1.0 μm or 1.6 μm (5 μg DNA per mg of gold particles) according to the procedures recommended by the manufacturer of Helios™ Gene Gun System (Biorad, Hercules, CA). The tubing coated with gold was cut into 0.5-inch segments. Thus, each segment contains 0.5 mg gold and 2.5 μg of reporter DNA.

All animal experiments were performed according to the ARVO resolution on the use of animals in vision research. New Zealand white rabbits (about 2 kg) were anesthetized with ketamine (30 mg/kg) and xylazine (3 mg/kg). One drop of 0.5 % proparacaine/HCl was applied to cornea.[30] Back hair was clipped and residual hair was removed by treatment with NAIR® (Carter-Wallace, NY). The Gene Gun was held against corneas, conjunctivas and skin to bombard gold particles into tissues, 1 delivery per individual cornea, 2 deliveries onto opposite sites of individual conjunctiva, and up to 12 sites on the skin of individual rabbits. Samples were collected 48 h after delivery and subjected to further experiments.

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,ooo x g, 4°C for 10 min.

Assays of -galactosidae Activity 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. 0.7 ml 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 KB reporter gene constructs was calculated as a fold-increase of the promoter-less pNASSβ construct.

The whole eye ball was enucleated, fixed immediately with 4% paraformaldehyde in PBS at 4°C for 2hr, and then washed three times with PBS at 4°C. Staining was carried out at 30°C for 16 hr in a solution of 5-bromo-4-chloro-3-indolyl-β-galactopyronoside (X-gal, Sigma) at a final concentration of 0.4 mg/ml made from a 40 mg/ml stock in dimethylformamide, with 4 mM $K_3Fe(CN)_6$, 4 mM $K_4Fe(CN)_6.6H_2O$, 2 mM $MgCl_2$ in PBS. After staining, eyeballs were rinsed with PBS and photographed as whole mounts.

Preparation of Nuclear Extracts

Nuclear extracts were prepared as described by Andrews and Faller.[31] Briefly, bovine corneal epithelialcells were scraped into ice-cold PBS, and homogenized in lysis buffer (0.1% Titon X-100). Then homogenized solution was purified through 1.8 M sucrose cushion at 24,000 rpm for 45 mins, and the pellet was resuspended in cold buffer (20 mM HEPES-KOH, pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM dithiothreitol, 0.2 mM PMSF). Then, it was homogenized with dounce-glass homogenizer, and incubated on ice for 20 min. Cellular debris was removed by centrifugation for 2 min at 4 °C. Then the supernatant fraction, containing nuclear DNA-binding proteins, was precipitated by 0.39 g/ml of NH_4 OH at 34,000 rpm for 25 mins and stored in aliquots at 70 °C.

DNase I Footprinting Aanalysis.

Four DNA probes (-332 bp to –109 bp, -394 bp to –131 bp, -599 bp to –375 bp, -1415 bp to –1394 bp) were prepared with polymerase chain reaction by four pairs of primers (Table 2), and labeled with [γ-^{32}P] ATP by T4 polynucleotide kinase at the 5'-end. They were incubated with crude nuclear extracts (75 μg) from bovine corneal epithelial cells as previously described.[32] A 50 μl mixture containing 10 mM Tris (pH 8), 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 incubated for additional 45 minutes with 100,000 CPM of DNA probe before DNase I digestion for 1 minutes and 30 seconds at 25 ^{o}C with variable amount of DNase I. The DNase I digestion was stopped with 50 µl of stop buffer (0.2 M NaCl, 30 mM ethylenediaminetetraacetic acid, 1% sodium dodecyl sulfate, and 0.1 mg/ml yeast tRNA). Digested DNA probes were purified by phenol extraction and ethanol precipitation and separated on 6.5% denatured 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 (probe) were performed in the absence of nuclear protein extracts. Region protected by nuclear proteins and numbers indicating nucleotide positions relative to the transcription start site of the K12 gene.

Electrophoretic Mobility Shift Assays.

Gel-shift analysis of different region of *Krt1.12* promoter was performed with 20000 CPM of ^{32}P-labeled double-stranded synthetic oligonucleotide probe (-182 bp to –111 bp, -256 bp to –293 bp, –942 bp to –913 bp, -1661 bp to –1624 bp, and –1887 bp to –1858 bp) (Table 3), and crude nuclear extracts prepared from bovine corneal epithelial cells as described. Twenty microliters of binding reaction mixtures contained 10 µg of nuclear proteins, end-labeled [γ-^{32}P] double strand oligonucelotides (20000 CPM), 12

mM HEPES (pH 7.9), 10% glycerol, 4 mM Tris (pH 8.0), 1 mM ethylenediaminetetraacetic acid, 0.3 mg/ml bovine serum albumin, 1mM dithiothreitol, 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.

Results

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

To identify *cis*-acting regulatory elements in the 5'-flanking region of the *Krt1.12* gene, we constructed a series of 5' deletion β-gal expression vectors and transiently transfected them into rabbit conneal, conjunctival, and cutaneous epithelia by gene guns. The promoter activities of the various constructs obtained from 6 independent experiments in rabbit corneal epithelial cells are summarized in Fig. 1. Data obtained from β-gal activities of skins and conjunctivas (the same constructs as in Fig.1) were not significantly different from the activity of pNASSβ in conjunctival and cutaneous epithelia (data not shown).

The activity of pNASSβ construct was assigned a relative level of 1.0. As described above, 5.0 kb, 2.5 kb, and 2.0 kb fragments displayed similar β-gal activities, and were significantly more than the activity of pNASSβ (Student's *t*-test, $p<0.05$). Deletions of 445, 645, and 845 bp from –975 bp to 3' end respectively did significantly lower β-gal activities of 2.0 kb constructs (Student's *t*-test, $p<0.05$). 5' deletion of 402 and 600 bp from –1014 bp to 5'end in 2.5 kb constructs resulted in further decreasing in β-gal activity compared with (Student *t*-test, $p<0.05$), suggesting the presence of a enhancer-like element(s) between –1613 bp and –1014 bp, especially from –1416 bp to 1014 bp. However, further 5' deletion constructs from -1816 bp to –1014 bp and

from –2015 bp to –1014 bp, rescued some β-gal activities (Sudent's *t*-test, $p<0.05$). These results suggested that the region between –2015 bp and –1613 bp contains a silencer-like element(s).

We further tested three constructs, –527 bp to +40 bp, -327 bp to +40 bp and –127 bp to +40 bp (Fig. 2), to determine whether the presence of tissue-specific elements existed between –527 bp to +40 bp of *Krt1.12* promoter. We found that the 0.2 kb construct, -127 bp to +40 bp, did not express the tissue specificity (Fig 2. and Fig. 3A). However, the tissue specificity could be found in 0.4 kb (-327 bp to +40 bp) and 0.6 kb constructs (- 527 bp to +40 bp) (Fig. 2, Fig 3B. and Fig 3C). We suggested that there might be a tissue-specific element(s) in the region from –327 bp to +40 bp.

DNase I Footprinting Assay

Four probes (-1415 bp to –1167 bp , -599 to-375 bp, -394 bp to –131 bp, and –331 bp to –109 bp) were used to identify the possible transcriptional factor binding sites according to results functional analysis in the promoter of *krt1.12* gene. There was two continuous binding regions from –182 bp to –110 bp and from –256 bp to –193 bp could be found in the DNase I binding assays (Fig.4). There was no binding site could be found in another two probes (~-1415 bp to –1167 bp, -599 bp to-375 bp). From these results, we suggested that the fragment from –182 bp to –110 bp might be responsible

for some fundamental promoter activities of *Krt1.12* gene, but this region was not for the tissue specificity. However, the fragment form –256 bp to –193 bp might play an important role of tissue specificity, and could also be found the constructs longer than 0.4 KB (-375 bp to +40 bp).

Electrophoretic Mobility Shift Assays (EMSA)

To characterize transcription factors that interact with the binding sites in the *Krt1.12* promoter, EMSAs was performed. Synthetic oligonucleotides used in EMSAs (Table 3) were designed from the regions protected by nuclear proteins. EMSA of site from –182 to -111 and –256 to –193 showed binding complex with nuclear proteins from nuclear proteins of corneal epithelial cells, but the band could also be detected with nuclear proteins from retinal cells (Fig. 5) This complex was competed and eliminated by a 400- or 800-fold molar excess of the unlabeled oligonucleotides termed self-competitor. These results suggested that there were several transcriptional factors acting with oliogonucleotides and regulating the tissue specificity of *Krt1.12* promoter.

In the regard of PAX-6 elements, three segments of synthetic probe corresponding to the –942 bp to –913 bp, -1661 bp to –1624 bp, and -1887 bp to –1858 bp respectively, were used to test the EMSAs. We observed that the first segment gave a nonspecific binding with nuclear extracts, the second segment could bind the nuclear extracts and eliminate their binding with nonlabeled probe, abd the third segment didn't

bind the nucelar extracts (Fig. 6).

Discussion

A 0.4 kb Krt1.12 Promoter can express Corneal Specificity

In this paper, we showed those constructs including 5.0 kb, 2.5 kb, 2.0 kb, 0.6 kb, 0.4 kb, and 0.2 kb of Krt1.12 promoter displayed similar activities on corneal epithelial cells (Fig. 1 and Fig. 2). Except the 0.2 kb construct, all of them expressed minimal activities on conjunctival and cutaneous epithelial cells. This result strongly suggests that a 0.2 kb of Krt1.12 promoter can efficiently express the promoter activities in epithelial cells and is consistent with our previous findings.[30] However, this 0.2 kb construct could not display corneal specificity (Fig. 2 and Fig. 3). The corneal specificity could only occur in constructs longer than 0.2 kb. We further used deletion constructs to confirm the tissue specificity. When we deleted 0.4 kb, 0.6 kb, and 0.8 kb from 1.0 kb in a 2.0 kb of Krt1.12 construct, we observed that the promoter activities decreased gradually and the corneal specificity disappeared in the 0.8 kb deletion construct (Fig. 1 and Fig 3). This further confirmed the region from 0.2 kb to 0.4 kb could regulate the expression of corneal specificity and implied there also exists some enhancer elements from 0.2 kb to 1.0 kb.

DNase I Footprinting and EMSAs

To further elucidate the actual transcriptional binding sites in these regions, DNase I footprinting assay was performed. We found that there were two fragments (-256 bp

to –193 bp and –182 bp to –111 bp) could be protected by nuclear extracts and these two fragments could also be confirmed by EMSAs. However we also found that retinal nuclear extracts could also bind both segments. Combining above data, we suggested that these two segments might be responsible for the expression of corneal tissue specificity and the sequence proximal to 3' end of these regions played a role in the regulation of *Krt1.12* promoter activity in epithelial cells.

By searching the Gene Bank (NCBI GenBank database, Blast web client software), we found that there were eight possible transcriptional factor binding sites including AP-1 (-240 bp to –250 bp), VPB (-229 bp to -238 bp), CP-2 (-211 bp to -221 bp), S8 (-158 bp to -174 bp), GFI1 (-114 bp to -138 bp), AP1 (-124 bp to -133 bp), DELTAFF1 (-125 bp to -136 bp), AP4 (-113 bp to -122 bp) binding site in sense direction of these areas. In the antisense orientation, we also found there were eleven possible transcriptonal binding sites including AP4 (-113 bp to -122 bp), PADS (-123 bp to -132 bp), OCT1 (-128 bp to -153 bp), CEBPB (-133 bp to -147 bp), OCT1 (-119 bp to -133 bp), CATA-1 (-218 bp to -231 bp), CEBP (-226 bp to -243 bp), CEBP B (-229 bp to -242 bp), GKLF (-234 bp to -248 bp), BARBIE (-236 bp to -251 bp), and GKLF (-239 bp to -253 bp) binding sites. From these binding sites, we speculated that the regulation of *Krt1.12* promoter in the expression of corneal specificity is the result of combination of several transcriptional factors in these regions, which was suggested

by previous studies of other cytokeratin genes.[21;22]

Among these transcriptional factors, AP-1 (activation protein 1) was well known to be a regulator of many cytokeratin genes including K1, K3, K5, K8, K10, and K18.[6] [33-37]The regulation may be a basal activity or response to stimuli, e.g. calcium, vitamin D, and steroid hormone, through the protein kinase C (PKC) or mitogen activated protein kinase (MAPK) pathways.[38-42] But the roles of other transcriptional factors in the regulation of cytokeratin gene were still unknown.

Form the deletion analysis, we suggested that there were enhancer element(s) from –975 bp to –534 bp and –1416 bp to –1014 bp. Probes (-1415 bp to –1167 bp and -599 bp to-375 bp) were also performed to study the binding with nuclear extracts, but we still could not find any protection region in these probes. We don't know what kind of transcriptional factors in these areas due to too broad to be analyzed by specific probes in EMSAs. But from our previous studies, we found that co-transfection of Pax-6 cDNA with K12 promoter-β-gal constructs result in 4-fold increase of β-gal activities.[30]Other studies also demonstrated that Pax-6 played an important role in the regulation of eye-specific gene in lens, iris epithelium, and retina.[43-45]We constructed three probes as described above for EMSAs. We found that the probe from –1661 bp to –1624 bp, which contains two Pax-6 binding sites, could specifically bind the corneal nuclear extracts. A high level of Pax-6 is known to be maintained in adult corneal

epithelium, but also can be expressed in other ocular tissue.[46] We suggested that this Pax-6 segment enhanced the promoter activity of K12 gene, but might be not involved in the regulation of corneal specificity.

In conclusion, we found the upstream sequence (-182 bp to –111 bp and –256 bp to –193 bp) of *Krt1.12* promoter contains the capability to regulate the corneal specificity. A minimal 110 bp (0.2 kb) of *Krt1.12* promoter can efficiently drive the basal activity in corneal and conjunctival epithelial cells. The regulation of corneal specificity might be from corporation of several transciptional factors. The Pax-6 element enhanced the promoter activity, but not corneal specificity.

Table 1. Plasmid Constructs Used in Gene Gun Assay and Probes Used for Footprinting

	K12 Promoter Length (bp)	Deletion Sequence (bp)	Sense Primer	Antisense Primer
5.0 KB	(-5034~-+40)			
2.5 KB	(–2501 ~ +40)			
2.0 KB	(–2001 ~ +40)			
0.6 KB	(–527 ~ +40)		GGAATTCCC AGATGCTCTC AGAGCC	TTTGCACCA CCACCGGA T
0.4 KB	(–327 ~ +40)		GGAATTCCTT CGGGAAGGA TTCCTTA	TTTGCACCA CCACCGGA T
0.2 KB	(–127 ~ +40)		GGAATTCCTC ACATACCTGC TGAGCT	TTTGCACCA CCACCGGA T
2 KB-0.6 KB	(–2001 ~ +40)	(–975 ~ -534)		
2 KB-0.4 KB	(–2001 ~ +40)	(–975 ~ -334)		
2 KB-0.2 KB	(–2001 ~ +40)	(–975 ~ -134)		
2.5 KB-1.0KB	(–2501 ~ +40)	(–1416 ~ -1014)	GGAAGAAAT AACCAGGAC AGGGGCTGG AG	GTAAGAAA TACCCAGG ACAGGGGC TGGAG
2.5 KB-1.0 KB	(–2501 ~ +40)	(–1613 ~ -1014)	GGAAGAAAT AACCAGGAC AGGGGCTGG	GAAAGAAA ACCCAGGA TCAAACAG ACTGGC
2.5 KB-1.0 KB	(–2501 ~ +40)	(–1826 ~ -1014)	GGAAGAAAT AACCAGGAC AGGGGCTGG	TGTAAATAT ATGCCTGG GAAGCACC AGCAA
2.5 KB-1.0 KB	(–2501 ~ +40)	(–2015 ~ -1014)	GGAAGAAAT AACCAGGAC AGGGGCTGG	ACTGACATC AGTTCCCAT GTTCCTGCT GT

Table 2. Primers Used for Dnase I Footprinting

Probe Length (bp)	Sense Primer	Antisense Primer
-1415~-1167	CCCCTGTCCTG GGTATTTCTTA	GAGAGTTGGTCTCA AGGTCAGT
-599~-375	ACTTCCAAACA GCTGGCTC	CGGTTCAGTATAAG CCAACC
-394~-131	GGTTGGCTTATA CTGAACCG	TGCATCGTGAAAAT TCACTG
-331~-109	GGCTTCGGGAA GGATCCTT	CCAGCTCAGCAGGT ATGTGA

Table 3. Probes for Electrophoretic Motility Shift Assays

Probe Length (bp)	Sequence
-256~-193	ACTGAAGGTGACAGATTCCTTACGACAGCCTA
	TCTGCTCCACCCAGCCTTCTTTCTTGTGTGTC
-182~-111	TGGTAATGGTTATTCGATTATAGCTATATCAGTGAAT
	TTTCACGATGCATAAATCACATACCTGCTGAGCTG
Pax 6 (-1887~-1858)	AAATGAGGCAAGTGGATTGCAGACTGTGT
Pax 6 (-1661~-1624)	TTTTCAAACACTTTCCCAGGGTCAGGAACA
Pax 6 (-942~-913)	CGTTTTATTAAATTCCTGTGAATTCTTTGG

Figure 1.

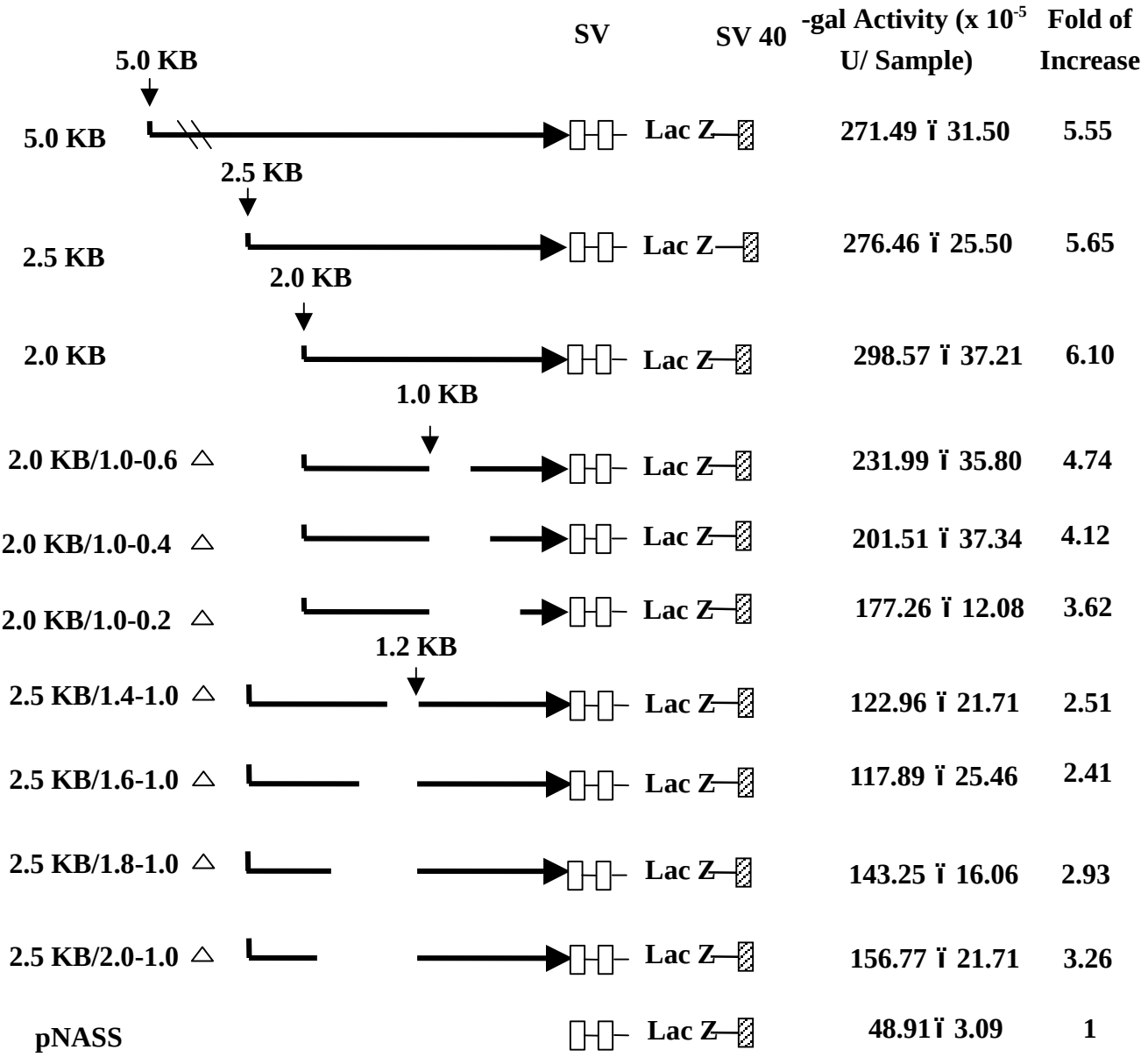
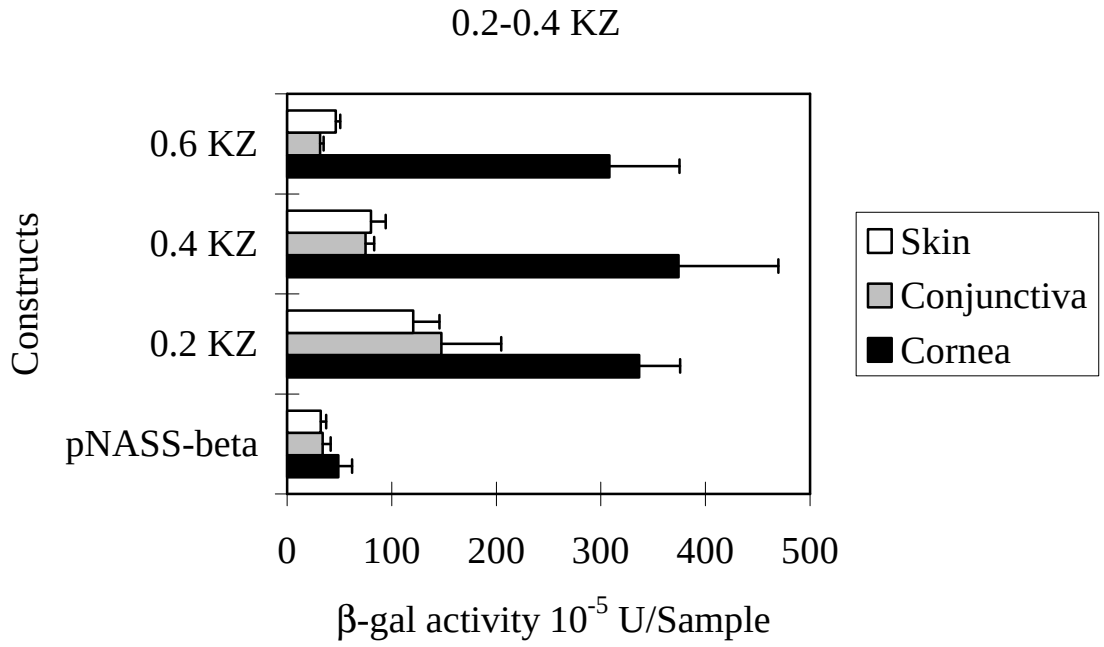

SV
SV 40
-gal Activity (x 10-5 U/ Sample)
Fold of Increase
5.0 KB
2.5 KB
2.0 KB
1.0 KB
1.2 KB
5.0 KB
Lac Z
271.49 ï 31.50
5.55
2.5 KB
Lac Z
276.46 ï 25.50
5.65
2.0 KB
Lac Z
298.57 ï 37.21
6.10
2.0 KB/1.0-0.6 △
Lac Z
231.99 ï 35.80
4.74
2.0 KB/1.0-0.4 △
Lac Z
201.51 ï 37.34
4.12
2.0 KB/1.0-0.2 △
Lac Z
177.26 ï 12.08
3.62
2.5 KB/1.4-1.0 △
Lac Z
122.96 ï 21.71
2.51
2.5 KB/1.6-1.0 △
Lac Z
117.89 ï 25.46
2.41
2.5 KB/1.8-1.0 △
Lac Z
143.25 ï 16.06
2.93
2.5 KB/2.0-1.0 △
Lac Z
156.77 ï 21.71
3.26
pNASS
Lac Z
48.91ï 3.09
1

Figure 2.


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

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