The maturation of stratified squamous epithelium of the upper gastrointestinal tract is a highly ordered process of development and differentiation. Information on the molecular basis of this process is, however, limited. Here we report the identification of the first murine forestomach regulatory element using the murine adenosine deaminase (Ada) gene as a model. In the adult mouse, Ada is highly expressed in the terminally differentiated epithelial layer of upper gastrointestinal tract tissues. The data reported here represent the identification and detailed analysis of a 1.1-kb fragment that is sufficient to target cat reporter gene expression to the forestomach in transgenic mice. This 1.1-kb fragment is capable of directing cat reporter gene expression mainly to the forestomach of transgenic mice, with a level comparable to the endogenous Ada gene. This expression is localized to the appropriate cell types, confers copy number dependence, and shows the same developmental regulation. Mutational analysis revealed the functional importance of multiple transcription factor-binding sites.

The mucosa of murine upper gastrointestinal tract tissues, i.e. tongue, esophagus, and forestomach, like the epidermis, undergoes organized progressive differentiation to form a mature stratified squamous epithelium, which is comprised almost exclusively of keratinocytes (1–3). Keratinoctye differentiation is the process whereby a relatively undifferentiated keratinocyte in the basal layer is converted, via intermediate spinous and granular layers, into a terminally differentiated cornocyte in the cornified layer (4, 5). Insights into transcriptional regulation of gene expression in keratinocytes have been gained mainly through analysis of the eukaryotic cytokeratin promoters (6–11), and to a lesser extent, the cornified envelope precursor genes in skin or in epidermal epithelial cell lines (12–23). Genes expressed in the epidermis appear to be lineage-specific. For example, keratins 5 and 14 are expressed in the proliferating basal layers. In the differentiated suprabasal layer, their expression is down-regulated, as the expression of early differentiation marker genes keratin 1 and 10 and involucrin are turned on. Loricrin, filaggrin, transglutaminase, localized in the upper spinous and granular layers of the epidermis and upper GI tract tissues, are predominant markers for late epithelial differentiation. Genes expressed in the squamous epithelium of the upper GI tract, despite limited research, have been shown to resemble the epidermis. In mouse forestomach, keratins 5 and 14 are expressed in the basal layers, while keratins 1 and 10 are expressed in the suprabasal layer where cells are terminally differentiated (24). Although some progress has been made in identifying and understanding factors that regulate gene expression in esophageal epithelium from investigation of the Epstein-Barr DNA virus (25–28), much work needs to be done. Moreover, while the majority of research has focused on keratin genes involved in basal cell proliferation or early differentiation, and the early differentiation marker involucrin gene, only a few of the several genes involved in the latest stages of epithelial differentiation have been studied, including transglutaminase 3, loricrin (20, 23), and small proline-rich protein 1A (29).

Adenosine deaminase (ADA) is a pivotal purine catabolic enzyme that converts adenosine and deoxyadenosine to inosine and deoxyinosine, respectively (30). It is ubiquitously distributed among vertebrate tissues, but the level of expression varies markedly among different tissues. In humans, high levels of ADA are found in the proximal small intestine and thymus. The upper GI tract tissues, especially the stomach, also express enhanced levels of ADA (31). In adult mice, the highest levels of ADA also occur in the gastrointestinal tract, including the absorptive epithelium of the small intestine and the keratinized squamous epithelium that lines the tongue, esophagus, and forestomach. In fact, these mucosal layers are the richest naturally occurring sources of ADA where the enzyme accounts for as much as 20% of the total soluble protein (32). Immunostaining analysis showed that ADA is localized predominantly to the granular layer of keratinized epithelium (32), rendering it another marker of the late stage of keratinocyte differentiation in the upper GI tract. Given the abundance of murine ADA and its cellular localization in the upper GI tract, utilizing the Ada gene as a model for studying gene expression in the GI tract will likely contribute to our knowledge of keratinocyte terminal differentiation, and enhance our appreciation for the function of the vast amount of ADA in the GI epithelium. The work presented in this paper focuses on defining the cds-regulatory elements and protein factors that direct this enhanced level of expression in the GI epithelium, especially the forestomach epithelium.

Using transgenic mouse techniques, we have identified a
1.1-kb Egl-I-HindIII fragment from 5′-flanking region of the murine Ada gene that is able to direct chloramphenicol acetyltransferase (CAT) gene expression in the forestomach of transgenic mice. In situ hybridization shows that the cat expression directed by the forestomach regulatory element exhibits the same cellular localization as the endogenous Ada gene. This cat expression also displays copy number dependence, shows the same developmental timing and comparable level as the endogenous Ada gene. Sequence analysis revealed both general and tissue-specific protein-binding sites within this 1.1-kb sequence. Deletions and site-specific mutations of some potential protein-binding sites resulted in decreased levels of reporter gene expression, suggesting the involvement of these factors in regulating Ada expression in the forestomach.

MATERIALS AND METHODS

Plasmid Construction—The 6.4CAT transgene, described by Winston et al. (33) as the construct ADACAT, was subcloned into the BamHI site of Bluescript KS+ II vector (Stratagene). Deletions were prepared by appropriate restriction digestion of this parental plasmid. 3.5FCAT and pCAT were digested by HindIII and XbaI digestion of 6.4CAT, respectively. 2.0FCAT, 1.1FCAT, 0.9FCAT, 0.6FCAT, and 0.5FCAT were generated by ligation of the pCAT construct to various restriction fragments from the 6.4-kb Ada flanking sequence. To generate AP2FCAT, a pair of complimentary oligonucleotides CGGC(TC)C(AAGG)TGGTGC-ACTATGGTGTGCACTATG and GCTCTAGAAGCTTCTAGAGC and GCTCTAGAAGCTTCTAGAGC and GCTCTAGAGC were generated. These oligonucleotides were cloned into the pCAT vector for sequencing. Transcription factor consensus sequences were sought using the Findpatterns program both from the Genetics Computer Group (GCC) program and Online MatInspector (35).

RESULTS

A Forestomach-specific Regulatory Element Resides within a 1.1-kb Fragment in the 5′ Flank of the Murine Ada Gene—We have shown previously that a 6.4-kb region immediately upstream of the murine Ada gene is capable of directing the expression of a reporter gene to the placenta prenatally and to the forestomach postnatally (33). We sought to identify the forestomach-specific regulatory element by deletion analysis in transgenic mice. Transgenic mice were used because this approach provides a physiological assay system in which the complete array of necessary regulatory elements can be defined in a developmental context. To increase the pace of the analysis, we chose to study founder mice instead of generating transgenic lines since the same features of cat expression were observed in both assays. Of five 6.4CAT transgenic lines, four showed high levels of CAT activity in the forestomach and undetectable CAT activity in the adjoining hindstomach and small intestine. All seven 6.4CAT founders showed high levels of forestomach-specific cat expression. The level of cat expression also correlated with copy number (Table I). Thus, the possibility of transgenic mosaicity did not present a problem, and transgenic founders worked as well as the transgenic lines to define the Ada forestomach regulatory element.

We tested various 6.4CAT deletion constructs for their ability to target cat expression in the forestomach of founder adult mice (Fig. 1). Deletion to a HindIII site (3.3FCAT) disrupted expression, while a 2.0-kb BamHI-EglI fragment at the 5′-half (2.0FCAT) gave no detectable expression. The 3′-1.1-kb EglI-HindIII fragment fused to the cat reporter cassette (1.1FCAT) restored forestomach expression, and the expression level was similar to that of the original 6.4-kb fragment. Other adult tissues in which high level Ada expression is normally found did not show consistent expression of cat, although the tongue sometimes gave about 100 times lower levels of cat expression (Fig. 2). Further deletion from either 5′ (0.6FCAT) or 3′ (0.5FCAT) end of this fragment led to a drastic decrease of cat expression in the forestomach (Fig. 1). Therefore, the 1.1-kb EglI-HindIII fragment, as the first recognized forestomach regulatory element, retains all the information required for

| Transgenic line | CAT activity | CAT activity per copy | Transgene copy number |
|-----------------|--------------|-----------------------|-----------------------|
| Line 1          | 52           | 39,000                | 780                   |
| 66              | 19,700       | 1,200                 | 17                    |
| 19              | 9,900        | 1,100                 | 7                     |
| 77              | 260          | 260                   | 1                     |
| 68              | 260          | 260                   | 1                     |

Table I: Comparison of CAT specific activities in adult forestomachs of 6.4 CAT transgenic lines and founders

| Founder | CAT activity | CAT activity per copy | Transgene copy number |
|---------|--------------|-----------------------|-----------------------|
| 2717    | 4,690        | 670                   | 7                     |
| 3266    | 5,220        | 870                   | 6                     |
| 2720    | 3,100        | 620                   | 5                     |
| 3267    | 3,300        | 1,100                 | 3                     |
| 3268    | 2,100        | 700                   | 3                     |
| 3265    | 1,880        | 840                   | 2                     |
| 3255    | 1,080        | 540                   | 2                     |
high level forestomach-specific expression of the cat reporter gene.

The Ada Forestomach Regulatory Element Directs Reporter Gene Expression to the Same Cellular Localization as the Endogenous Ada Gene—To confirm that cat gene expression occurred in the same cell types as the endogenous Ada gene, the cellular localization of cat transcripts from the 1.1FCAT transgene was identified by in situ hybridization. The forestomach tissue from a transgenic founder containing approximately 14 copies of the 1.1-kb transgene was sectioned, fixed, and then hybridized with either cat or Ada sense or antisense 35S-labeled UTP RNA probes. Both cat and Ada antisense probes produced strong signals in the keratinized squamous epithelial cell layer. Neither Ada nor cat mRNA was detected in the adjacent muscle layer of the forestomach (Fig. 3) or the adjacent hindstomach tissue (data not shown). Thus, this 1.1-kb EagI-HindIII fragment contains information necessary for appropriate cellular localization of cat reporter gene expression.

The Ada Forestomach Regulatory Element Directs Reporter Gene Expression with the Appropriate Developmental Timing—In gastrointestinal tissues, the level of ADA protein is subject to pronounced developmental control, being low at birth and achieving enormous levels within the first 5 weeks of postnatal life. To determine whether the developmental timing of cat expression coincided with forestomach Ada gene expression during epithelial layer formation, the early onset of cat expression was compared with that of ada expression in the epithelial layer. CAT activity was measured in the forestomach of transgenic mice at birth, at 10 days, and at approximately 4 months. CAT activity was low at birth, but by weaning, CAT was highly expressed in the forestomach, and significant expression appeared by 4 months (about 120 days) of age (Fig. 4). In situ hybridization also showed that the expression is localized only in the epithelial layer of the forestomach (data not shown). From these data, we conclude that this 1.1-kb EagI-HindIII fragment contains sufficient genetic information for reproducing the endogenous developmental pattern of Ada gene expression in the forestomach.

The Forestomach Enhancer Directs Reporter Gene Expression in a Copy Number-dependent Manner—To examine whether this 1.1-kb EagI-HindIII fragment allows a position-independent, copy number-dependent expression of the reporter gene, the level of cat expression in the forestomach of each transgenic founder mouse was compared with its copy number. This comparison revealed a linear relationship between the level of cat expression in each transgenic mouse and its transgene copy number.
number (Fig. 5). The forestomach regulatory element therefore functions in an integration site independent and copy number-dependent manner.

The Forestomach Enhancer Is Capable of Directing cat Expression in the Transgenic Forestomach in a Level Similar to the Endogenous Ada Gene—To determine whether the quantity of cat expression in the transgenic forestomach was comparable to that of endogenous Ada expression, the respective levels of each steady state message were compared in gastrointestinal tissues, including tongue, esophagus, forestomach, hindstomach, and small intestine. RNA was isolated from these tissues, and the ratio of cat message to Ada message in the forestomach was determined by RNase protection assay using $^{32}$P-labeled cDNA probes for both Ada and cat genes. While Ada message was observed in tongue, esophagus, forestomach, and small intestine, consistent with the observed pattern of enzyme activity, cat message was detectable only in the forestomach. When adjusted for gene dosage, the magnitude of cat expression per transgene in the forestomach was similar to the endogenous Ada gene. Thus, the forestomach regulatory element is capable of delivering reporter gene expression to the transgenic forestomach at a high level, being comparable to endogenous Ada genes (Fig. 6A).

Because cat transcripts and endogenous Ada transcripts may differ in their stability, a forestomach enhancer-driven Ada minigene (1.1FADA) was constructed and transgenic mice were generated. This minigene contained the endogenous last intron, poly(A) signal and 3’-untranslated region to ensure similar processing and stability as the endogenous gene (36). A 36-bp deletion at the 5’-untranslated region was engineered to allow the transgene mRNA to be easily distinguished from the endogenous mRNA (Fig. 6B). Total RNA was extracted from tissues of gastrointestinal tracts in different F0 mice carrying the 1.1FADA. RNase protection assays were performed on these tissues using the same Ada probe for the 1.1FCAT mice. The protected band for endogenous Ada was 310-bp, while that for minigene is 275-bp (Fig. 6B). The ratios in several transgenic founder mice were close to 1 when adjusted to transgene
copy numbers (Table II). It thus indicates that this forestomach enhancer is capable of directing high level expression of the Ada minigene as well as the cat reporter gene in the forestomach, indicating that it may contain most, if not all, the genetic information required for the expression of Ada gene in the forestomach.

Deletion of 200-bp 5’ Region and the Mutation of a Putative AP-2 Factor-binding Site Results in Decreased CAT Expression in the Transgenic Forestomach—A data base search was performed to identify potential transcription factor-binding sites within this 1.1-kb forestomach regulatory element. The sequence analysis revealed multiple candidates (Fig. 7) which have been shown to be of importance in regulating gene expression in epidermal as well as esophageal epithelium. Among these are a cluster of AP-1, C/EBP, and CACCC-like binding motifs within the 5’ 200-bp of the forestomach element, and an AP-2 factor consensus site at the 3’ end. Deletion of the 200-bp 5’ region resulted in a drastic decrease of the expression level of cat reporter gene, indicating the functional importance of this region (Fig. 8). Similarly, a two-base mutation of CC to TT at the AP-2 site led to almost total diminution of activity, strongly indicating the importance of this site in the enhancement of forestomach expression (Fig. 8). Therefore, mutational analysis suggests that multiple factors in the forestomach epithelium may act through the forestomach regulatory element.

**DISCUSSION**

In addition to its low level of ubiquitous expression in almost all tissues, the murine Ada gene is expressed at substantially elevated levels in a small collection of diverse tissues, especially in those of the gastrointestinal tract. The expression of Ada in the GI tract displays a cell type- and lineage-specificity, confined to the epithelial cells lining the mucosa of the tongue, esophagus, forestomach as well as small intestine (32). The relative level of ADA protein is extremely high, comprising approximately 20% of soluble protein in the mucosal layer (32). To facilitate a greater understanding of the physiological significance of ADA in the gastrointestinal tract and the regulation of gene expression in the upper GI tract, we wish to identify signaling pathways that govern the temporal and cellular expression of the murine Ada gene during GI development using transgenic mouse assays. The data reported here represent the identification and detailed analysis of a 1.1-kb sequence located 3.4 kb upstream of the transcription initiation site of the murine Ada gene, which is sufficient to target cat reporter gene expression to the forestomach in transgenic mice. This expression is localized to the appropriate cell types, confers copy number dependence, and shows the same developmental regulation. These facts indicate that this forestomach-specific regulatory element contains all the cis-acting genetic information that is necessary to reproduce the endogenous pattern of murine Ada expression in the forestomach.

Both immunofluorescence staining from previous work in our laboratory (32) and *in situ* hybridization in the present study showed that murine Ada message is localized predominantly to the keratinized stratified squamous epithelium, especially to the late differentiated keratinocytes. Ada is undetectable in any non-squamous cells in the lamina propria or muscularis mucosae in the mucosa, submucosa, external muscularis mucosae, or serosa. The identified forestomach enhancer is capable of directing cat reporter gene expression mainly to the terminally differentiated cells. Detailed analysis of this 1.1-kb regulatory element in the forestomach epithelium is therefore of importance, since it will give us insights into the molecular basis of gene regulation on the terminally differentiated cells in the upper GI epithelium.

Although tongue and esophagus are histologically similar to forestomach, CAT assays as well as RNase protection assays showed that this forestomach regulatory element does not direct enhanced cat expression in those tissues, at least not at expected high levels as compared with those of the endogenous Ada expression. These interesting observations, along with a number of previous studies both in human and mouse, indicate that the regulation of murine Ada in each tissue is complex and involves different regulatory elements for different or even closely related tissues or cell types, in this case, all the upper GI tract tissues. In fact, we and others have shown that distinct regulatory modules govern expression in both the murine thymus (37) and placenta (38) as well as the human thymus (39) and small intestine (40). In addition, a locus control region has also been identified in intron 1 region of the human ADA gene (39, 41), and similarly, an element responsible for ubiquitous expression in intron 1 of the murine Ada gene (37).

The position independence, copy number dependence associated with transgenes carrying the forestomach regulatory region, and the strong enhancer activity observed in the transgenic forestomach qualify this element as locus control region (LCR), a regulatory element acting as a dominant activator in establishing the transcriptional competency of a complete gene.
Since their initial description in the human β-globin locus, LCR elements have been recognized in several other genes, including the human ADA T-cell enhancer/LCR and duodenal enhancer (40, 41). Unlike human T-cell/LCR and duodenal enhancer, the identified forestomach regulatory element neither drives ubiquitous low level expression, nor requires additional sequences for full activation. It, in fact, seems to contain all the information to ensure integration site independent, copy number dependent expression of transgenes. LCRs have been suggested to exert their functions by recruiting protein factors that lead to hyperacetylation of histones and subsequently to the unfolding of the chromatin (42–44). The forestomach regulatory element presumably contains multiple binding sites for sequence-specific transcriptional activators for its function both as an enhancer and as an LCR, and it may exert its LCR function by recruiting histone acetyltransferase, such as CBP/p300 (45), to the Ada promoter. The hyperacetylation of histones and subsequent unfolding of the chromatin may eventually result in activation of the Ada promoter. Further analysis of this element would give us more insight regarding how LCR elements function.

To fully understand the molecular aspect of the regulation of this forestomach enhancer, we conducted mutational analysis of this enhancer to identify important transcription factor-binding sites. In the process of determining protein factors involved in regulating this forestomach enhancer using footprinting and gel mobility shift assays, we encountered some difficulties. Since the keratinized stratified squamous epithelium where Ada is highly expressed in the forestomach is terminally differentiated and enucleated, it is very difficult to prepare nuclear extract from the forestomach mucosa. However, sequence analysis of this enhancer revealed several potential transcription factor-binding sites that have been shown to be functionally important on other keratinocyte-specific promoters or enhancers, among which is an AP-2

![FIG. 6. Comparison of the level of cat expression in construct 1.1FCAT and a forestomach enhancer driven Ada minigene with that of the endogenous Ada gene. A, comparison of cat expression with endogenous Ada gene expression. 1.1FCAT transgene construct is shown above and includes the 1.1-kb forestomach enhancer (dotted box), 800-bp murine Ada promoter (shaded oval), cat cDNA (white box), SV40 small antigen intron region and polyadenylation site (hatched box). 30 μg of total RNA isolated from tissues of a 1.1FCAT founder was incubated with a mixture of uniformly radiolabeled 280-bp cat and 410-bp Ada probes. The cat probe protects a 270-bp fragment, while the Ada probe protects a 310-bp fragments. B, comparison of expression of the forestomach enhancer driven Ada minigene with that of the endogenous Ada gene. The forestomach enhancer driven Ada minigene is shown above and includes the murine Ada cDNA (boxed), forestomach enhancer (dotted box), 800-bp murine Ada promoter (shaded oval), SV40 small antigen intron region and polyadenylation site (hatched box). 30 μg of total RNA isolated from tissues of a 1.1FCAT founder was incubated with a mixture of uniformly radiolabeled 280-bp cat and 410-bp Ada probes. The cat probe protects a 270-bp fragment, while the Ada probe protects a 310-bp fragments.]

**TABLE II**

| Founder | Minigene/endogenous gene mRNA ratio | Ratio adjusted for copy number |
|---------|-----------------------------------|-------------------------------|
| 5076    | 1.21                              | 1.86                          |
| 5077    | 1.7                               | 0.76                          |
| 5078    | 3.4                               | 1.36                          |
| 5086    | 1.9                               | 1.09                          |

locus/chromatin domain (42). Since their initial description in the human β-globin locus, LCR elements have been recognized in several other genes, including the human ADA T-cell enhancer/LCR and duodenal enhancer (40, 41). Unlike human T-cell/LCR and duodenal enhancer, the identified forestomach regulatory element neither drives ubiquitous low level expression, nor requires additional sequences for full activation. It, in fact, seems to contain all the information to ensure integration

![FIG. 7. Sequence of the Ada forestomach regulatory element. The sequence of the 1.1-kb Egr1 to HindIII fragment is shown with key restriction sites marked by lowercase letters. The sequence motifs for AP-1, C/EBP, CACC-like, and AP-2 are shown boldfaced and underlined.]

site independent, copy number dependent expression of transgenes. LCRs have been suggested to exert their functions by recruiting protein factors that lead to hyperacetylation of histones and subsequently to the unfolding of the chromatin (42–44). The forestomach regulatory element presumably contains multiple binding sites for sequence-specific transcriptional activators for its function both as an enhancer and as an LCR, and it may exert its LCR function by recruiting histone acetyltransferase, such as CBP/p300 (45), to the Ada promoter. The hyperacetylation of histones and subsequent unfolding of the chromatin may eventually result in activation of the Ada promoter. Further analysis of this element would give us more insight regarding how LCR elements function.

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factor-binding site located at the 3' end of this regulatory element. AP-2 consensus binding motifs have been recognized in many keratin promoters and enhancers and other epidermis-specific or squamous epithelium-specific genes (46, 47). Despite this, it has been shown that AP-2 determines, in vitro, the level of transcription rather than functioning as a sole determinant of the epithelial specificity of gene expression (48). Similar to their findings, the cat reporter gene expression in our AP-2 mutant construct (AP2FCAT) decreased significantly, however, forestomach restricted expression remained (data not shown). This result indicates that the role of the AP-2-like factor in squamous epithelium gene expression is quantitative rather than qualitative. That is, rather than determining epithelial specificity, this AP-2-like factor modulates, in vivo, the level of expression of epithelial genes. AP-2 contains three isoforms AP-2α, AP-2β, and A-2γ, all of which have distinct but partially overlapping patterns of expression (49–53). Our RNase protection assays (data not shown) and data from other laboratories (51–53) confirmed the presence of all three AP-2 isoforms in the upper GI tract tissues, which suggest that AP-2α, AP-2β, and A-2γ may all be involved in the regulation of this forestomach enhancer. Further transfection analysis is needed to delineate the definite role of each AP-2 gene in regulating this enhancer and, moreover, squamous epithelium genes in general.

Within a 200-bp region at the 5' end of the forestomach enhancer, there is a cluster of transcriptional binding motifs that have been shown to regulate the expression of other squamous epithelium-specific genes. These motifs include six AP-1 sites, two CCAAT/enhancer-binding proteins (C/EBP) binding sites and a CACCC-like motif. AP-1 binding motifs are found in the keratin promoters (11, 54) and other early or late differentiation stage genes (19, 21, 55), and are important determinants of the keratinocyte stage of differentiation (23). C/EBP proteins, members of the bZIP family of DNA-binding proteins/transcription factors, play a fundamental role both in the differentiation of adipocytes to adipocytes and in the regulation of the expression of many different genes encoding cytokines in several cell types (56–60). High level expression of C/EBP has recently been shown to be associated with squamous differentiation in epidermis, suggesting that it may play an important role in regulating one or more aspects of the squamous epithelium differentiation program (61, 62). The importance of the CACCC-like element in the transactivation of genes expressed in the upper GI tract has been demonstrated in its interaction with gut-enriched Krüppel-like factor and activating the Epstein-Barr virus EL-2D promoter (26, 27). Gut-enriched Krüppel-like factor is localized to suprabasal cells in skin, tongue, esophageal, and intestinal squamous epithelial cells (63), rendering it a good candidate for regulating the suprabasal-layer expressed Ada gene. Although the role of each possible transcription factor has not been assessed, the functional importance of this 200-bp region is evident since deletion of this sequence abolished reporter gene activity in the transgenic forestomach. Additional experiments are required to fully assess the importance of the above mentioned transcription factors.

The evidence that the Ada forestomach enhancer consists of a collection of distinct genetic regulatory motifs suggests that a combination of multiple transcription factors are required for the forestomach epithelium-specific expression of murine Ada, even though many of these factors, such as AP1 and AP-2, also appear in nonepithelial cells. It is possible that appropriate combinations of these potential factors, including AP-2 factor, AP1 factors, C/EBP factors, gut-enriched Krüppel-like factor, and other ubiquitously as well as epithelium-specific factors, may provide a mechanism for the determination of epithelium specificity (64). Future experiments will provide more insight into this aspect of the molecular mechanism of gene regulation in the squamous epithelium.

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