Inhibition of Lens Fiber Cell Morphogenesis by Expression of a Mutant SV40 Large T Antigen That Binds CREB-binding Protein/p300 but Not pRb*

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Simian virus (SV) 40 large T antigen can both induce tumors and inhibit cellular differentiation. It is not clear whether these cellular changes are synonymous, sequential, or distinct responses to the protein. T antigen is known to bind to p53, to the retinoblastoma (Rb) family of tumor suppressor proteins, and to other cellular proteins such as p300 family members. To test whether SV40 large T antigen inhibits cellular differentiation in vivo in the absence of cell cycle induction, we generated transgenic mice that express in the lens a mutant version of the early region of SV40. This mutant, which we term E107KΔ, has a deletion that eliminates synthesis of small t antigen and a point mutation (E107K) that results in loss of the ability to bind to Rb family members. At embryonic day 15.5 (E15.5), the transgenic lenses show dramatic defects in lens fiber cell differentiation. The fiber cells become post-mitotic, but do not elongate properly. The cells show a dramatic reduction in expression of their β- and γ-crystallins. Because CBP and p300 are co-activators for crystallin gene expression, we assayed for interactions between E107KΔ and CBP/p300. Our studies demonstrate that cellular differentiation can be inhibited by SV40 large T antigen in the absence of pRb inactivation, and that interaction of large T antigen with CBP/p300 may be enhanced by a mutation that eliminates the binding to pRb.

DNA tumor viruses such as simian virus 40 (SV40) and adenovirus inactivate pivotal checkpoints for cell cycle control, thereby stimulating host cell DNA synthesis and allowing viral DNA replication. The early region of SV40 encodes two proteins: the large T and small t antigens. Large T antigen is a potent 708-amino acid oncoprotein, which has transforming ability and can induce tumors in animal models (1). Relevant targets of T antigen include the retinoblastoma family members, pRB, p107, and p130, which interact with the amino-terminal domain of T antigen (amino acid residues 102–114). The central and carboxyl-terminal regions of T antigen bind to the tumor suppressor p53 and to a variety of other proteins including the transcriptional co-activators CREB-binding protein (CBP), p300 and p400 (1, 2). In certain cell types, such as lens fiber cells, preadipocytes, and myoblasts, expression of large T antigen is sufficient to stimulate cell cycle entry and cellular transformation (3–8).

The ocular lens provides an excellent model system in which to study the relationship between cell cycle exit and differentiation-specific gene expression. The lens is composed of an anterior monolayer of proliferative cuboidal epithelial stem cells overlaying a core of terminally differentiated, post-mitotic, elongated fiber cells. At the equatorial region of the lens, epithelial cells are induced to exit from the cell cycle and to differentiate into fiber cells. Initiation of fiber cell differentiation is accompanied by up-regulation of expression of the cyclin-dependent kinase inhibitor Kip2 (p57) (9), and activation of expression of fiber cell-specific proteins including the β- and γ-crystallins (10, 11). These changes in gene expression reflect alterations in the activities of specific transcription factors including c-Maf, Sox-1, and Prox-1 (12–14). Recent in vivo and in vitro studies indicate that transcriptional coactivators CBP/ p300 are required for lens fiber cell differentiation and expression of crystallin genes (15, 16).

CBP and p300 are distinct but functionally related coactivator proteins with histone acetyltransferase activity (17). Both proteins have been shown to interact with sequence-specific transcription factors, including CREB (18), c-Fos (19), c-Jun (20), c-Myb (21), E2F1 (22), and p53 (23). CBP and p300 also interact with viral proteins such as the adenovirus E1A protein (24), SV40 large T antigen (2), human papillomavirus E6 protein (25), and polyomavirus large T antigen (26). These viral proteins can bind to and inhibit both CBP and p300. In the ocular lens, we have shown that CBP and/or p300 are important coactivators of c-Maf-mediated transcription from various crystallin promoters (16). Although it has been reported that lens-specific expression of wild type SV40 large T antigen causes lens tumors and reduced expression of crystallins (3), little is known about whether inhibition of crystallin expression by SV40 T antigen is solely because of loss of pRb activity and cell cycle re-entry (27), or because of direct inhibition of other essential endogenous proteins. In this study, we have expressed in the lens a mutant version of SV40 large T antigen, E107KΔ, which does not bind to the pRb family members. We show that the E107KΔ protein can inhibit both fiber cell differentiation and crystallin gene expression without altering cell cycle regulation. We also show that the E107KΔ protein

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¶ The abbreviations used are: CREB, cAMP-response element-binding protein; CBP, CREB-binding protein; PBS, phosphate-buffered saline; BrdUard, 5-bromo-2′-deoxyuridine.
binds to CBP/p300 and blocks interaction of CBP/p300 with c-Maf.

EXPERIMENTAL PROCEDURES

Generation of the Constructs and Transgenic Mice—The entire early region of SV40, encoding small t and large T antigens, was previously linked to the α-crystallin promoter (Fig. 1A) (3). For the E107KΔ construct, a BamHI/BglII fragment from plasmid cTruncT268-E107K (4) was substituted for the corresponding region of the wild type clone (Fig. 1C). This fragment contains both a deletion of nucleotides 4586–4854 (Δ268) to remove the small t splice donor, and the K1 mutation (2, 6, 28) (University of Wisconsin, Madison, WI) that eliminates binding of T antigen to p73. The K1 mutation replaces glutamic acid (E) with lysine (K) at amino acid 107 (E107K) in the LXXE motif (2). The resulting plasmid was digested with KpnI and EcoRI to release a 2.5-kb fragment for microinjection (Fig. 1C). The truncated T antigen construct (Fig. 1B) has been described previously (4). The fragments used for microinjection were separated by electrophoresis through a 1% agarose gel, and purified using a GeneClean kit (Bio 101 Inc., Vista, CA). Transgenic mice were generated by pronuclear injection of the purified fragments into one-cell stage inbred FVB/N embryos (29, 30).

Immunohistochemistry and Immunofluorescence—Tissue sections (5-μm thick sections) were deparaffinized in xylene and rehydrated through a graded alcohol series, washed with PBS, and incubated with 10% methanol, 3% hydrogen peroxide in PBS to block endogenous peroxidase activity. For detection of α-crystallins, rabbit antisera (from Samuel Zigler, National Institutes of Health) were used at 1:1000 dilution in 10% normal horse serum in PBS, with a 24-h incubation at 4 °C. After washing with PBS, biotinylated goat anti-rabbit IgG was applied for 45 min at 37 °C. The secondary antibody was detected using streptavidin-conjugated horseradish peroxidase, and peroxidase activity was visualized with diaminobenzidine (Vector Laboratories, kit SK-4100). All slides were counterstained with hematoxylin.

Apoptosis detection kit (apoTACS TM: In Situ Hybridization) was used to perform TUNEL assay using an in situ apoptosis detection kit (apopTACS). In Situ Apoptosis Detection Kit, Trevigen, Inc.). Tissue sections from embryos at E15.5 were treated as described (4, 15).

Western Blots—Total proteins from prenatal eyes or newborn lenses of transgenic and non-transgenic (FVB) mice were isolated. Tissue was sonicated (level 4, 30%, 25 pulses) in lysis buffer containing 150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, protease inhibitors (Mixture tablets, from Roche Diagnostics), 1 mM EDTA, and 5 mM EGTA. After centrifugation at 16,000 × g for 4 °C for 20 min, the supernatants were collected and the protein concentration was determined using the Bio-Rad protein assay kit (Bio-Rad). 20 μg of total lens or eye protein were denatured in 2× SDS sample buffer and boiled for 5 min. The proteins were separated by SDS-PAGE, and transferred onto polyvinylidene difluoride (DuPont) membranes. The blots were blocked with 5% nonfat dry milk (Carnation) in TBS (Tris-buffered saline, 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.02% Tween 20) at 4 °C overnight. The blots were then incubated with the primary antibody (anti-α-crystallin, 1:500, anti-β-crystallin, 1:200, anti-γ-crystallin, 1:200, all from Samuel Zigler; anti-c-Maf, 1:500, from Santa Cruz; anti-T antigen, 1:500, from BD Biosciences), rabbit polyclonal anti-c-Maf (1:500) (Santa Cruz), mouse monoclonal anti-c-Maf (1:500) (Sigma), and rabbit or mouse IgG (1:500) (Vector Laboratories) were added and incubated overnight at room temperature. The slides were washed with PBS (4 × 5 min), and a secondary antibody (1:200) (biotin-conjugated anti-rabbit or anti-mouse IgG, Vector Laboratories) was added for 1 h at room temperature. After washing 4 times with PBS, ExtrAvidin-fluorescein isothiocyanate conjugate (from Sigma) was added and incubated for 1 h at room temperature, followed by PBS washes. The slides were mounted with Aqua-Poly/Mount (Vector Laboratories).

Detection of Apoptosis—DNA fragmentation was detected by TUNEL assay using an in situ apoptosis detection kit (apopTACS). In Situ Apoptosis Detection Kit, Trevigen, Inc.). Tissue sections from embryos at E15.5 were treated as described (4, 15).

RESULTS

Microphthalmia in E107KΔ Transgenic Mice—Previous studies have shown that expression of SV40 large T antigen in
lens fiber cells induces cell cycle entry dependent upon the ability of T antigen to bind to the tumor suppressor pRb, and that cell cycle re-entry is accompanied by defects in fiber cell elongation and a reduction in β- and γ-crystallin expression (4). To analyze whether T antigen can inhibit cell morphogenesis, elongation and a reduction in ability of T antigen to bind to the tumor suppressor pRb, and lens fiber cells induces cell cycle entry dependent upon the presence of multiple extra nuclei, indicative of cell cycle re-entry. Pyknotic nuclei (indicative of cell death) are visible in the truncated T antigen lens (4). B, D, F, H, and J are higher magnifications of the lenses in A, C, E, G, and I. Abbreviations: co, cornea; le, lens epithelium; nr, neuronal retina. Scale bars = 500 μm.

**Table I**

| Version of T antigen | Binds to pRb? | Induces S phase in fiber cells? | Induces apoptosis in fiber cells? | Blocks to CBP/p300 expression? | Blocks crystallin expression? |
|----------------------|---------------|-------------------------------|---------------------------------|-------------------------------|-------------------------------|
| Wild type            | Yes           | Yes                           | No                              | Yes                           | Partial                       |
| Trunc.T              | Yes           | Yes                           | No                              | No                            | Partial                       |
| E107KΔ               | No            | No                            | No                              | Yes                           | Almost completely |

**Fig. 2. Inhibition of fiber cell morphogenesis.** Ocular histology at E15.5 is shown for the following genotypes: non-transgenic FVB (A and B), E107KΔ (OVE481 (C and D) and OVE484 (E and F)), wild type T antigen (OVE266, G and H), and truncated T (OVE272, I and J). Lens fiber cells (f) in a normal lens (A) are elongated, post-mitotic, highly eosinophilic, and generally span the entire central diameter of the lens from the posterior lens capsule to the inner margin of the anterior epithelial cells (B). The E107KΔ transgenic mice have small lens morphogenesis (incomplete elongation), detached cells in the core of the lens and anteriorization of the transition zone (compare arrows in B, D, and F). OVE266 and OVE272 mice at E15.5 (G–I) show alterations in fiber cell elongation and alignment accompanied by the presence of multiple extra nuclei, indicative of cell cycle re-entry. Pyknotic nuclei (indicative of cell death) are visible in the truncated T antigen lens (4). B, D, F, H, and J are higher magnifications of the lenses in A, C, E, G, and I. Abbreviations: co, cornea; le, lens epithelium; nr, neuronal retina. Scale bars = 500 μm.

**Fig. 3.** E107KΔ expression does not induce fiber cell proliferation or apoptosis. Expression of E107KΔ mRNA was detected using a 35S-labeled riboprobe for section in situ hybridizations to E15.5 non-transgenic (FVB) (A), transgenic OVE481 (B) and OVE484 (C) eyes. Hybridization signals were captured as dark-field images. To detect the transgenic protein, immunostaining was done using an antibody against SV40 large T antigen (D–F). E107KΔ expression (green stain) was detected in lens epithelial and fiber cells in both transgenic families (E and F). The protein localized to the nucleus. BrdUrd incorporation was assayed by immunostaining at E15.5 (G–I). BrdUrd-positive cells (brown nuclear stain) are restricted to the epithelial (e) cells in the wild-type lens (G), and to the anterior of the lens in both transgenic families (H and I). TUNEL assays were used to assay for cell death in lenses from FVB (J), OVE481 (K), and OVE272 (L) embryos. There were many apoptotic fiber cells (brown nuclear stain) in OVE272 lenses (L). No apoptosis was detected in either FVB (J) or OVE481 (K) lenses. Arrows in G–I indicate the transition zones from where the proliferating lens epithelial cells become post-mitotic fiber cells. Scale bar = 500 μm.

**Lens Histology.—**Transgenic lenses expressing E107KΔ are small (Fig. 2), and are partially hollow because of failure of primary fiber cell elongation. The small lenses show anteriorization of the differentiation zone (arrows in Fig. 2, D and F).
The E107KΔ phenotype resembles that seen in c-Maf or Sox-1 knockout mice (13, 36). Our prediction when we started these experiments was that the mice would have normal lenses. Because the mice exhibited a dramatic phenotype, we have studied them to characterize the pattern of transgene expression, and to assay for alterations in cell cycle regulation, fiber cell differentiation, and expression of crystallin genes.

Expression of E107KΔ in Lenses of Transgenic Mice—Based on in situ hybridizations (Fig. 3, B and C) and immunostaining using an antibody specifically against SV40 large T antigen (Fig. 3, E and F), expression of the E107KΔ transgene was lens-specific. Hybridization signals (Fig. 3, B and C) and green nuclear staining for SV40 large T antigen (Fig. 3, E and F) were found in lens epithelial and fiber cells in both transgenic families (OVE481 and OVE484). The expression of the transgene in lens epithelial cells was not typical for the αA-crystallin promoter, and might be related to the small size of the lens. In non-transgenic lenses, cell proliferation is normally confined to the epithelial cells that cover the anterior of the lens (Fig. 3G). The epithelial cells are normally induced to become post-mitotic fiber cells at the equatorial region. Lens-specific expression of either wild type or truncated SV40 T antigen, or targeted mutagenesis of the Rb gene, causes the post-mitotic lens fiber cells to re-enter S phase (4, 27). In contrast, posterior lens fiber cells expressing E107KΔ did not incorporate BrdUrd (Fig. 3, H and J). This result is consistent with the prediction that E107KΔ is unable to bind to pRb. It also implies that E107KΔ does not disrupt fiber cell differentiation by inducing cell cycle re-entry (Table I). TUNEL assays were performed to test for cell death in the different transgenic lenses (Table I and Fig. 3, J–L). There were no detectable apoptotic nuclei in the lens fiber cells expressing E107KΔ (Fig. 3K). Thus, the altered differentiation in E107KΔ transgenic lenses is not because of inappropriate induction of cell death.

Expression of E2F1 and Cyclins—E2F1 is an important transcription factor that can activate expression of genes required for the G1/S transition (37). In normal mouse lenses, E2F1 is expressed in both epithelial and fiber cells (38). In contrast, expression of cyclins A2 and B1 in the lens is confined to the lens epithelial cells (Fig. 4, D and G). These cyclins are also expressed in proliferating retinoblasts (Fig. 4, D and G). Expression of full-length T antigen in lens fiber cells resulted in induction of expression of these cell cycle genes (Fig. 4, C, F, I, and L) (15, 31). By comparison, there was no detectable up-regulation of these genes in lens fiber cells expressing E107KΔ (Fig. 4, B, E, H, and K).

Expression of p57—The expression of p57 (Kip2), a cyclin-dependent kinase inhibitor, is strongly up-regulated when fiber cell differentiation is induced in the equatorial region of the lens (Fig. 4M, arrows). Enhanced p57 expression is required for the cell cycle exit that accompanies fiber cell differentiation (9). In the E107KΔ transgenic lenses, p57 expression was still up-regulated during fiber cell induction (Fig. 4N, arrows). Induction of p57 also occurred in lens cells expressing full-length T antigen (Fig. 4Q). These results suggest that inhibition of fiber cell morphogenesis by E107KΔ takes place after fiber cell differentiation has been initiated.

Expression of Other Lens Fiber Cell Differentiation Markers—Previous studies have shown that lens fiber cells without functional pRb have reduced expression of α-, β-, and γ-crystallins (3, 4), markers for lens cell differentiation. We used immunohistochemistry to test for α-crystallin expression, and Western blotting to assay for changes in expression of α-, β-, and γ-crystallins. We compared non-transgenic lenses to lenses expressing wild type T antigen (OVE266A), truncated T antigen (OVE272), or E107KΔ (OVE481) (Fig. 5). Expression of α-crystallin was detected in lens cells in the presence of wild type T (Fig. 5, B and E), truncated T antigen (Fig. 5, C and E), or E107KΔ (Fig. 5, D and E), although expression was significantly reduced when compared with non-transgenic FVB lenses (Fig. 5, A and E). Expression of β- and γ-crystallins was also decreased about 30–40% in the presence of either wild type T or truncated T antigen (Fig. 5, F and G). By comparison, lenses expressing E107KΔ showed a truly dramatic loss of β- and γ-crystallin expression (Fig. 5, F, lanes 10–12 and G; Table I).

E107KΔ Does Not Block Expression of c-Maf and CBP/p300—Recent studies indicate that transcriptional co-activators CBP and/or p300 are required for c-Maf-mediated transactivation of β- and γ-crystallin promoters during lens fiber cell differentiation (16). Therefore, the lens defects induced by E107KΔ expression might be because of inhibition of c-Maf or CBP/p300 expression or function. To test for inhibition of c-Maf expression, in situ hybridizations and Western blots were performed (Fig. 6). In non-transgenic FVB lenses (Fig. 6A), transcripts of c-Maf are up-regulated in post-mitotic lens cells at the equatorial region. Transgenic mice expressing either wild type T antigen or E107KΔ still showed lens-specific c-Maf mRNA expression (Fig. 6, B and C). Using Western blots, we found that the c-Maf protein was present in the E107KΔ transgenic lenses at levels comparable with the wild type T antigen lenses (Fig. 6D). To assay for effects of E107KΔ on c-Maf nuclear translocalization, immunofluorescence using an antibody against c-Maf protein was performed. As shown in Fig. 7, A–D, c-Maf proteins are normally present in cell nuclei of non-transgenic FVB lenses (Fig. 7A). Expression of wild type T antigen (Fig. 7D), or E107KΔ transgenic lenses (Fig. 7E), were stained with an antibody against c-Maf. In wild type T antigen lenses (Fig. 7D), c-Maf proteins were present in the nuclei of fiber cells. In E107KΔ transgenic lenses (Fig. 7E), c-Maf proteins were completely absent from the nuclei of fiber cells.
were considered to be significantly different for $p$ in non-transgenic FVB (A), wild type T antigen (B), truncated T (C), and mutant E107KΔ (D) transgenic lenses at E15.5. α-Crystallin expression was reduced but still detectable in lens cells expressing wild type T antigen (B), truncated T antigens (C), or E107KΔ (OVE481) (D). Scale bar = 500 µm. E, Western blots were used to quantify α-crystallin proteins in non-transgenic and transgenic lenses. Expression of actin was used as a loading control. F, Western blots were used to compare β- and γ-crystallin protein levels in newborn eyes from non-transgenic FVB (lanes 1–3), wild type T (lanes 4–6), truncated T (lanes 7–9), and E107KΔ transgenic eyes (lanes 10–12). The amount of protein loaded was: 20 µg in lanes 1, 4, 7, and 10; 40 µg in lanes 2, 5, 8, and 11; 80 µg in lanes 3, 6, 9, and 12. Actin was used as an internal control. G, blots were quantified using an imaging densitometer. Protein levels of β- or γ-crystallin were quantified by the ratio of blot density of crystallin to the density of actin. Results are the mean ± S.D. from three individual experiments. Statistical analysis was done using two-tailed Student’s t test using Microsoft Excel software. Data were considered to be significantly different for $p < 0.05$.

**DIAGRAM**

**Fig. 5. Dramatic inhibition of β- and γ-crystallin expression.** A–D, immunohistochemistry was used to test for expression of α-crystallin in non-transgenic FVB (A), wild type T antigen (B), truncated T (C), and mutant E107KΔ (D) transgenic lenses at E15.5. α-Crystallin expression was reduced but still detectable in lens cells expressing wild type T antigen (B), truncated T antigens (C), or E107KΔ (OVE481) (D). Scale bar = 500 µm. E, Western blots were used to quantify α-crystallin proteins in non-transgenic and transgenic lenses. Expression of actin was used as a loading control. F, Western blots were used to compare β- and γ-crystallin protein levels in newborn eyes from non-transgenic FVB (lanes 1–3), wild type T (lanes 4–6), truncated T (lanes 7–9), and E107KΔ transgenic eyes (lanes 10–12). The amount of protein loaded was: 20 µg in lanes 1, 4, 7, and 10; 40 µg in lanes 2, 5, 8, and 11; 80 µg in lanes 3, 6, 9, and 12. Actin was used as an internal control. G, blots were quantified using an imaging densitometer. Protein levels of β- or γ-crystallin were quantified by the ratio of blot density of crystallin to the density of actin. Results are the mean ± S.D. from three individual experiments. Statistical analysis was done using two-tailed Student’s t test using Microsoft Excel software. Data were considered to be significantly different for $p < 0.05$.

**DISCUSSION**

Terminal differentiation in the ocular lens as well as other systems is blocked by expression of the SV40 early genes (3–7). Suppression of adipocyte differentiation by SV40 large T antigen has previously been shown to occur independent of pRb family binding and small t action (5, 6). However, the mechanisms by which T antigen blocks differentiation for different cell types have not been established. We have used transgenic mice to characterize the dramatic biological activity of E107KΔ, a point mutant of SV40 large T antigen that no longer binds to pRb. In this study, we show that E107KΔ can function as a potent repressor of morphogenesis and of crystallin expression during terminal differentiation of lens cells. Although it is possible that inhibition of lens cell differentiation may involve various activities of T antigen, our results indicate that
selectively disrupts the interaction of co-activators CBP/p300 with the transcription factor c-Maf. Because a truncated version of E107K does not disrupt this interaction and does not inhibit morphogenesis, and because CBP/p300 activity is required for normal fiber cell differentiation (16), we predict that this function of E107K plays a central role in its ability to inhibit fiber cell differentiation and morphogenesis.

**Crystallin Expression**

*Requirement for CBP/p300*—The β- and γ-crystallin families of proteins normally undergo a dramatic induction during maturation of lens fiber cells. Expression of these genes begins after the fiber cells have permanently exited from the cell cycle. Multiple genes in each family are coordinately regulated. The coordinate regulation and strong levels of expression suggest that a small set of trans-activating nuclear proteins configure the promoters of these genes for maximal expression. Our results indicate that the E107K mutant can inactivate the pathway or process by which all of these genes are up-regulated. It does so without binding to pRb family members, without inducing cell cycle re-entry, without disrupting the initiation of differentiation, and without inducing apoptosis. Lack of crystallin induction correlates with inhibition of CBP/p300 binding to c-Maf. As a result, CBP and p300 are presumably no longer recruited by c-Maf to the crystallin promoters, and expression of these genes is consequently disrupted.

*Requirement for Cell Cycle Exit*—When pRb is inactivated in lens fiber cells, either by targeted mutagenesis or by overexpression of SV40 large T antigen, expression of β-, γ-, and crystallins is reduced in conjunction with inappropriate cell cycle re-entry (3, 27, 31). The reduced expression of crystallins may simply be a consequence of cell proliferation in the absence of pRb function. However, pRb has been shown to play a direct role in regulation of muscle cell differentiation and muscle-
specific gene expression by acting as a cofactor for MyoD (40). Therefore, an alternative possibility is that pRb is a positive regulator of crystallin expression. In our studies, we discovered that E107KΔ can significantly inhibit expression of β- and γ-crystallins without inhibiting pRb function (Fig. 5). Although not conclusive, this finding would indicate that the reduced expression of crystallins in Rb−/− lenses or transgenic lenses expressing truncated T reflects an intrinsic incompatibility between cell cycle progression and the high levels of crystallin gene expression seen in terminally differentiated fiber cells.

E107KΔ: A Repressor of Crystallin Expression

E107KΔ inhibited fiber cell cycle morphogenesis and crystallin expression without inducing cell cycle re-entry or cell death (Fig. 3). Our previous studies showed that when the same E107K mutation was introduced into a truncated T antigen, which does not encode the COOH-terminal domains of T antigen, no inhibition of lens fiber cell differentiation or crystallin expression occurred (4). Therefore, the COOH terminus of E107KΔ is essential for its ability to inhibit differentiation and morphogenesis. This region of the protein is known to be required for interaction with the third C/H-rich domain of CBP and p300 (2, 39).

Other observations lead us to hypothesize that the E107KΔ mutation might result in a “gain of function” as well as a “loss of function.” First, we noted that the E107KΔ phenotype was dominant when E107KΔ mice were mated to full-length T antigen mice (data not shown). Second, we found that E107KΔ inhibited crystallin expression more efficiently than wild type SV40 large T antigen (see Fig. 5). Third, E107KΔ blocks the binding of e-Maf to CBP/p300, whereas wild type T antigen does not (Fig. 6F). Previous cell culture studies reported that both wild type T antigen and the K1 mutant (E107KΔ) bind to CBP/p300 at comparable levels (2, 39). However, it was also shown that deletion of residues 98–126 (mutant T50L7) resulted in the simultaneous loss of interaction with pRb and binding to CBP (2) indicating that the binding sites overlap. We hypothesize that the E107KΔ mutant interacts with CBP/p300 differently than wild-type T antigen.

In summary, our results indicate that the E107KΔ mutation can cause both a loss of function (loss of binding to pRb) and a gain of function (inhibition of CBP/p300 binding to e-Maf). It is not yet clear whether other functions of E107KΔ play additional role(s) in disrupting lens fiber cell morphogenesis. Because the molecular control of morphogenesis in vivo is not well understood, our transgenic mice provide a novel model system for future studies.

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