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Activation and repression sequences determine the lens-specific expression of the rat γD-crystallin gene

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ABSTRACT

Rat lens nuclear extracts contain a factor that binds to position −57 to −46 of the rat γD-crystallin promoter region. This factor protects the sequence 5′-CTGCCAA-CGCAG-3′ in a footprint analysis. Binding to this region is crucial for maximal promoter activity in rat lens cells, but this sequence was unable to act as an enhancer when cloned in front of a heterologous promoter. A region directly upstream from this activating sequence, between position −85 to −67, acts as a strong silencer of promoter activity in non-lens cells. This silencing effect is mediated by trans-acting factor(s). Our data provide evidence for two regulatory elements in rat γD-crystallin gene expression, an activating sequence active in lens cells and a silencing sequence active only in non-lens cells. The factor that binds to the activating sequence could be detected only in lens cells and may be a determinant of the lens-specific expression of the γ-crystallin genes.

INTRODUCTION

Crystallin genes code for abundant water-soluble structural proteins in the eye lens. Together these proteins can make up more than 90% of the total lens protein content. In rat, crystallins are encoded by three distinct groups of genes, the α-crystallin gene family and the closely related β- and γ-crystallin gene families (1). The lens-specific recognition of the crystallin genes is highly conserved in evolution. The rodent γ-crystallin gene promoters are recognized as lens-specific promoters even in non-mammalian organisms such as chicken and Xenopus (2–5), while sequence similarity can be detected in the αA-crystallin promoter regions from species as far distant as mouse and chicken (6). The evolutionary conservation of the lens-specific recognition of the γ-crystallin promoters suggests a similar conservation of regulatory sequence elements and factors that interact with these elements. However, mapping of the regulatory sequence elements in chicken and mouse lens cells showed a species-specific pattern of sequence recognition (2). A similar observation was reported for the αA-crystallin promoter (7). The contradiction between the evolutionary conservation of lens recognition and the apparent difference in the sequence elements that are recognized might be resolved if the trans-acting factors responsible for lens-specific expression are identified. More importantly, characterization of these factors would also provide insight into the molecular basis for lens-specific expression. In the search for such factors so far only a trans-acting factor for the mouse αA-crystallin gene has been characterized (8). This protein though, was shown to be ubiquitous and therefore not likely to be involved in tissue-specificity. Using bandshift assays, an activity was identified in chicken lens nuclear extracts that bound to the mouse γF-crystallin promoter directly upstream of the TATA box. The presence of this activity in lens extracts only, suggested that this factor might be involved in generating tissue-specificity (9). We show here that nuclear extracts of rat lenses contain a factor that specifically binds to a stretch of 12 nucleotides directly upstream of the TATA box of the γD gene, from −57 to −45. The tissue distribution of this factor is restricted as it could not be detected in retina or brain cells. Competition assays confirmed that binding of this protein is essential for activity of the rat γD promoter in rat lens cells. A region located slightly further upstream, around −73, does interact with factors detected only in non-lens cells, where this interaction functions to silence the promoter. Tissue specificity of expression of the γ-crystallin genes is thus obtained by lens-specific activation as well as non-lens specific silencing.

MATERIALS AND METHODS

Construction of rat γ-crystallin CAT constructs

CAT constructs containing 5′ upstream sequences of the rat γD-crystallin gene have been described previously (2). The truncated silencer sequence in pBLCAT2γΔ was made by linearizing the plasmid pBLCAT2γ with ApaI followed by treatment with mungbean nuclease and religation. The exact size of the deletion was determined by sequencing, using the dideoxy method, after subcloning to a M13mp vector. Synthetic complementary oligonucleotides 5′-CCTGCCAACGCAGCAGACCTCCTGC-3′ and 5′-GCAGGAGGTCTGCTGCGTTGGCAGG-3′ were cloned either in the blunt-ended SalI site in front of the thymidine kinase promoter of pBLCAT2 (10), or in the blunt-ended EcoRI site in front of the −44 to +45 γD-crystallin CAT construct.

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Primary cultures

Newborn rat lens epithelial cells were isolated and cultured essentially as described for mouse lens epithelial cells (2) using an equivalent of two lenses per 35 mm dish (COSTAR). Primary cultures of other newborn rat cells were obtained by trypsinization of the various organs followed by culturing under the same conditions as used for the lens cells.

Analysis of rat lens epithelial cell RNA

Total cellular RNA was purified from cultures (11) and subjected to Northern analysis. cDNA fragments, labelled as described (12) to a specific activity of 10^9 cpm/μg, were used as probe. Hybridizations were performed overnight at 45°C in 50% formamide/6×SSC/5×Denhardt's solution/100μg/ml denatured herring sperm DNA. Following hybridization filters were washed twice at 42°C in 0.2×SSC/0.1%SDS. Filters were exposed to X-ray film at −70°C overnight.

DNA transfection and CAT assay

Cells were cultured for a maximum of 7 days before transfection with calcium phosphate DNA co-precipitates (13). All transfections were done in duplo. Cells were harvested 48 hours after transfection and assayed for CAT activity as described (14). CAT activity was measured by densitometric scanning of the autoradiograms. The values were corrected for differences in cell density by measuring protein content of cell extracts (Bio-Rad protein assay).

Preparation of nuclear extracts

Nuclear extracts were made according a protocol modified from that of Schreiber et al. (15). Newborn rat lenses (twenty) or an equivalent amount of brain or retina tissue were isolated on ice and transferred to a tube containing 400 μl cold buffer A (10 mM HEPES pH 7.9/10 mM KCL/1 mM EDTA/1 mM DTT/1 mM PMSF) and homogenized. The tube was left on ice for 10

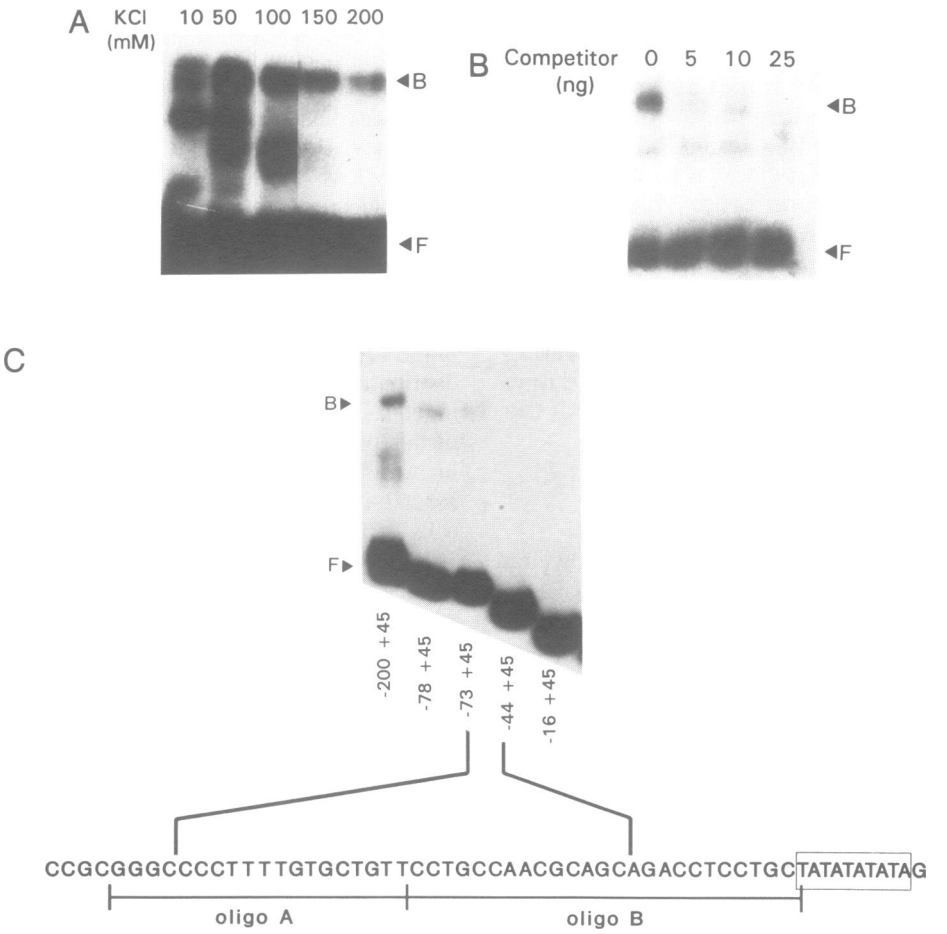

Figure 1. Complex formation of the γD-crystallin promoter region with rat lens nuclear factor(s). **(A)** Salt resistance of the complex. Bandshift assay were performed as described in Materials and Methods except that KCl concentration was varied as indicated. A labelled −200/+45 γD promoter fragment (0.1 ng) was used as probe. Free DNA (F) and the salt resistant high molecular band (B) are indicated. **(B)** Saturation of the complex. Bandshift assay were performed in the presence of increasing amounts of non-labelled −200/+45 γD promoter fragment. **(C)** Mapping of the binding region. (top) Labelled rat γD promoter restriction fragments containing the region indicated were incubated with nuclear extract from newborn rat lenses. The fuzzy bands in lane 1 beneath the indicated complex B were not always observed. Note that the signals from the −78 to +45 and −73 to +45 fragments, in which only a HindIII site has been filled in, are less intense due to the presence of only one label per molecule, while other fragments, in which both a HindIII and an EcoRI site have been filled in, have three labels per molecule. (bottom) Sequence of the corresponding region of the rat γD-crystallin promoter. The TATA box is indicated. The position of the double stranded oligonucleotides A and B (see text) is shown at the bottom.

min. after which 5 μl of NP40 was added. The nuclei were spun down at 1500 g for 3 min. The pellet was washed 3 times using 400 μl of cold buffer A to remove excess proteins (crystallins). Finally, the pellet was extracted by vigorous shaking for 10 min. at 4°C in 100 μl of cold buffer B (10 mM HEPES pH 7.9/500 mM NaCl/1 mM EDTA/1 mM PMSF). Cell debris was pelleted in a microfuge at 4°C and the supernatant was snap frozen in 10 μl aliquots before use.

Gel retardation assay

DNA restriction fragments containing either cloned γD-promoter fragments or cloned synthetic complementary oligonucleotides were end labelled by filling in sticky ends with Klenow DNA polymerase in the presence of α^{32}P-dATP. Approximately 0.1 ng of double stranded probe (10.000 to 20.000 cpm) was mixed with 0.5 to 3.0 μg of poly dIdC, to suppress non-specific binding, and 5−10 μg of nuclear extract in 20 μl of 20 mM HEPES pH 7.9/150 mM KCl/1 mM EDTA/1 mM DTT/4% Ficoll. Binding was allowed to proceed for 20 min. at room temperature after which the sample was loaded directly on a native 6% polyacrylamide gel which was prerun in 0.25×TBE for 30 min. at 1,5 Volt/cm. Gels were run for 2 hours with recirculation of the buffer, dried and exposed to X-ray film overnight without the use of intensifying screens.

In situ footprinting following gel retardation

Footprinting was carried out as described (16) using whole gels without the prior identification of retarded bands. After the DNA was nicked by exposure to a phenanthroline-copper ion solution, the bands were localized using short exposures (about 30 min.) and cut from the gel. DNA fragments were eluted overnight at 37°C in 500 μl of 500 mM ammonium acetate/1 mM EDTA. The eluted DNA fragments were isopropanol precipitated, resuspended in 10 μl of loading buffer, boiled for 3 min. and size-fractionated on a 9% sequencing gel. Gels were dried and exposed to X-ray film for several days using double intensifying screens (Dupont).

RESULTS

A nuclear factor binds to the sequence −57 to −46

In the search for trans-acting factors involved in the tissue-specific expression of the rat γ-crystallin genes a labelled γD-promoter fragment from −200 to +45 was assayed for its ability to specifically bind factors from lens nuclear extracts. As shown in Figure 1a, a number of complexes are formed when binding took place at 50 mM KCl. Only the high molecular weight band is resistant to higher salt concentration and remains when the KCl concentration is raised to 200 mM. This band represents a saturable complex as non-labelled −200/+45 fragments competed efficiently for binding (Figure 1b). The sequence specificity of the binding was tested by addition of increasing amounts of a non-specific competitor (poly dIdC). Even in the presence of 1 μg of poly dIdC a distinct band was observed (data not shown).

To localize more precisely the site of interaction of the factor within the promoter sequence, several labelled γD promoter fragments ranging from −200/+45 to −16/+45 were tested in the bandshift assay (Figure 1c; note that the specific activity of the −200/+45 probe is much higher, see figure legend). Fragments as short as −73/+45 were still capable of complex formation when incubated with rat lens nuclear extracts. Further shortening of the fragment to −44/+45 resulted in loss of factor binding capacity, indicating that the sequences responsible for complex formation are located between −73 and −44.

The DNA sequence between −73 and −44 can be divided into two separate domains. The first domain ranges from −73

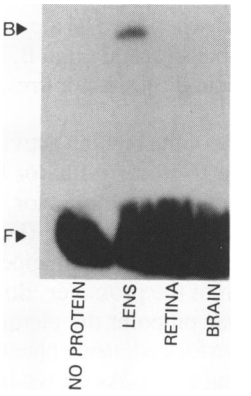

Figure 2. Lens-specificity of the nuclear binding factor. Radiolabelled double stranded oligonucleotide B was incubated with nuclear extracts either from newborn rat lens, retina or brain cells. Free (F) and bound (B) DNA fragments are indicated. Note that the same extracts were used in the experiments shown in figure 8c.

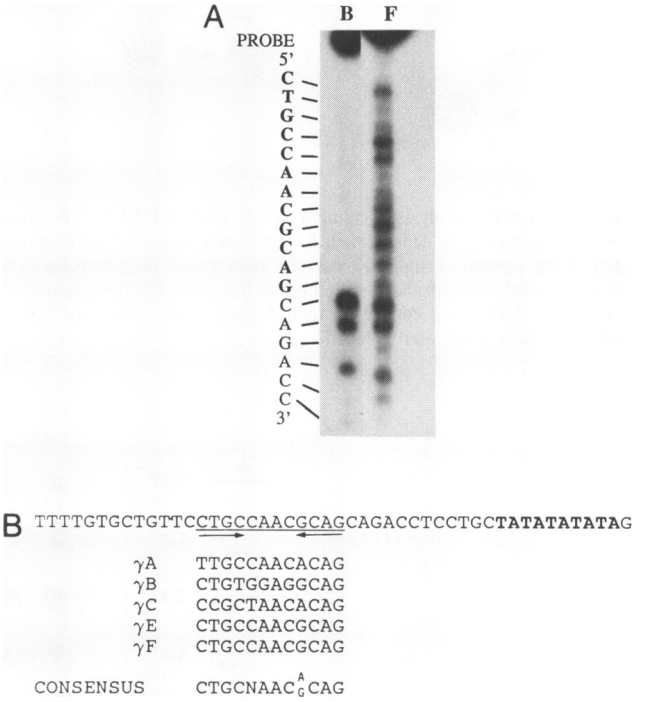

Figure 3. (A) Footprint analysis of the rat γD-crystallin gene promoter in the region −57 to −46. Radiolabelled double stranded oligo B was incubated with newborn rat lens nuclear extract and footprinted using phenanthroline-Cu, as described in Materials and Methods. Lane 1: bound probe, lane 2: unbound probe. The protected sequence is shown in bold to the left. **(B)** Conservation of the factor binding sequence in the rat γ-crystallin genes. The 12 nucleotides of the rat γD promoter protected by the lens nuclear factor are aligned with the corresponding sequences in the other rat γ-crystallin genes. The position of the factor binding site is underlined and the TATA box is shown in bold. The inverted repeat is indicated with arrows. A consensus sequence is shown at the bottom.

to −63 and is almost absolutely conserved in γ-crystallin promoters of rat, mouse and man (3,17−19); the second domain, between −63 and −44, is conserved as well but not to the same extent as the first domain. To examine to which of these two domains the factor was binding, two double stranded oligonucleotides were designed. Each of these oligonucleotides spanned a single domain (see Figure 1). Oligo A, which contained the first domain, was unable to bind any factor from rat lens nuclear extract when used as a probe in bandshift assays (not shown). The double stranded oligo B, containing the second domain, was indeed bound by a factor present in nuclear extracts from rat lenses.

The tissue specificity of the binding activity was tested by using nuclear extracts made from rat retina or brain. No binding to oligo B was found, suggesting the factor to be restricted in its distribution and possibly lens-specific (Figure 2).

The experiments described above mapped a binding sequence to the proximal region of the promoter, directly upstream of the TATA box. To further pinpoint the element recognized, an *in situ* footprinting was performed, using phenanthroline-copper ions (OP-Cu) as the cleaving agent. As shown in Figure 3a the factor protects the sequence 5′-CTGCCAACGCAG-3′ from −57 to −46. This sequence is also found in the promoter regions of the rat γE- and γF-crystallin genes, which are virtually identical to that of the rat γD gene, but is less well conserved in the promoter regions of the other rat γ-crystallin genes (Figure 3b). An alignment of these sequences yields the consensus CTGCNAAC-(A/G)CAG, which we have named the gamma-box. Interestingly, the gamma-box contains a inverted repeat, a characteristic of binding sequences of many trans-acting factors (see for example 20−22).

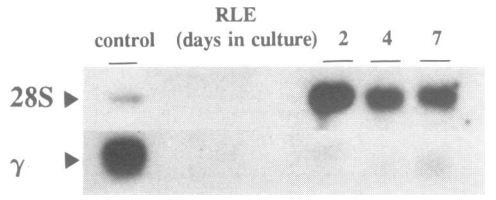

Figure 4. Northern blot analysis of cultured rat lens epithelial (RLE) cells. Total RNA was isolated from rat lens epithelial cells cultured for the length of time indicated. RNA (approx. 2 μg/lane) was size-fractionated on a formaldehyde agarose gel, blotted and hybridized with a probe for either 28S ribosomal RNA or a γ-crystallin RNA. As a control for hybridization a lane containing 100 ng of newborn rat lens RNA was included.

Binding to the gamma-box is required for activity of the γD-promoter in rat lens epithelial cells

To determine the functional relevance of the interaction of nuclear factors with the gamma-box for promoter activity, ideally a cell system is needed in which the activity of the γD promoter depends only on the proximal region. In the primary cultures of mouse lens cells, which rapidly differentiate to fibre cells *in vitro*, deletion of the upstream region to −73 already silenced the γD promoter (2). We had noted that newborn rat lens epithelial cells do retain their epithelial morphology in culture, suggesting that these cells do not differentiate under our *in vitro* conditions. The lack of differentiation of these cells was confirmed by analyzing the changes in γ-crystallin mRNA (a fibre cell specific transcript; 23) levels during culture of these cells. As shown in Figure 4, only traces of γ-crystallin mRNA could be detected in these cells and the level of γ-crystallin mRNA did not increase during the culture period. We argued that lens epithelial cells might have a different promoter recognition than lens fibre cells and therefore tested whether truncated γD promoter constructs were still active in these cells. A γD/CAT fusion gene containing only sequences up to −73 was as active as a γD/CAT fusion gene containing more than 1 kb of upstream sequences (Figure 5). Deletion of the region of −73 to −44, i.e. the region containing the gamma-box, did cause a decrease in the promoter activity to 10% of that of the full length clone. Removal of the TATA box and further downstream sequences (to −16) resulted in mere background activity, as expected.

To show that interaction of nuclear factors with the gamma-box is required for promoter activity, a competition experiment was performed in which the −73 to +45 γ-crystallin/CAT construct was co-transfected with either an excess of pUC19 containing oligo B, i.e. the gamma-box, or pUC19 without an insert. As a control these plasmids were also co-transfected with

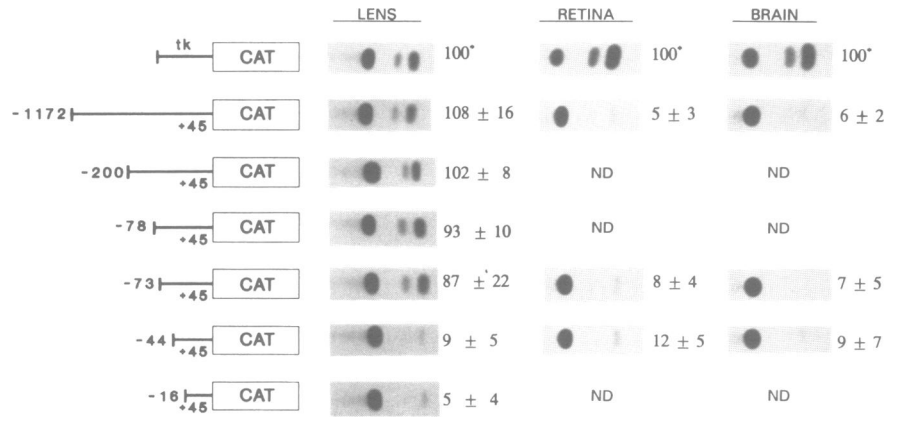

Figure 5. The activity of various rat γD promoter/CAT fusion constructs in primary cultures of rat lens, retina or brain cells. The region of the γD promoter contained in the CAT fusion genes is indicated. DNA was transfected and the CAT activity was determined as described in Materials and Methods. As a transfection control, pBLCAT2, containing the *Herpes simplex* virus thymidine kinase promoter, was transfected in parallel cultures. The CAT activity is given relative to that obtained from pBLCAT2 which was arbitrarily set at 100% (indicated by *). ND = not determined.

pBLCAT2, which contains the HSV-tk promoter driving the CAT gene. As shown in Figure 6a, competition with the gamma-box lowered the promoter activity of the −73 to +45 γD CAT construct with about 70%. No effect was detected on the activity of the HSV-tk promoter. This indicates that interaction of the gamma-box with nuclear trans-acting factors is required for activity of the γD promoter and shows that the gamma-box is indeed a regulatory element.

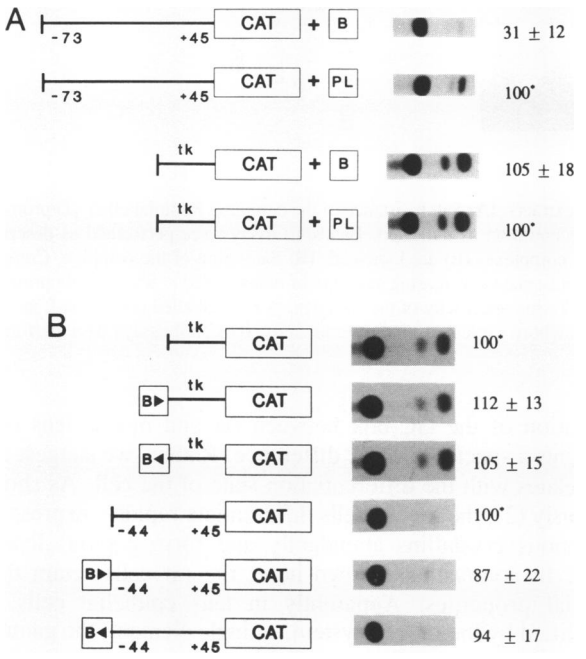

Figure 6. (A) Binding of the lens nuclear factor is essential for maximal promoter activity. The −73 to +45 rat γD-crystallin CAT construct or pBLCAT2 were co-transfected in cultured newborn rat lens cells with a fivefold excess of either pUC19 containing oligo B (B) or pUC19 (PL), and assayed for CAT activity. CAT activities were calculated relative to the activity obtained in the assays using the same CAT construct but pUC19 as the competitor (100*). **(B)** The effect of the −57 to −46 region on promoter activity. A single copy of the double stranded oligo B was cloned in two orientations in front of the HSV-tk promoter, or the truncated −73 to +45 γD-crystallin gene promoter. Constructs were transfected into newborn rat lens cells parallel with the parental plasmids and assayed for CAT activity. Activities are given relative to that of the parental constructs indicated as 100*.

The gamma-box does not act as an enhancer

Many DNA regulatory regions can act as enhancers. To test whether the gamma-box can act as a lens-specific enhancer, one copy of the double stranded oligo B, containing this sequence, was cloned in front of the HSV-tk promoter in the plasmid pBLCAT2. When transfected into rat lens epithelial cells neither orientation gave rise to a significant increase in CAT activity as compared to the HSV-tk promoter alone (Figure 6b). As the gamma-box was unable to enhance a heterologous promoter, we tested whether the sequence was an effective enhancer on its own promoter. The double stranded oligo B was therefore cloned in front of the truncated −44 to +45 γ-crystallin/CAT construct in an attempt to restore full promoter activity to this construct. The insertion of the sequences −57 to −46 in front of the truncated promoter did not result in restoration of promoter activity. In the 'reconstructed' γD promoter the gamma-box is located 25 bp upstream from TATA box, while in the natural situation the distance is only 12 bp upstream from the TATA box. Hence, these results could be explained by assuming that a close and critical distance to the TATA box is required for promoter activation by this element. Alternatively, the region between −73 and −57 might be required for promoter activity, although no interaction with nuclear factors by this region could be detected in the gel shift assay.

Silencing sequences within the region −85 to −67.

During our search for a rat tissue capable of recognizing a 'minimal' γD-crystallin promoter, we noted that none of the γD/CAT clones tested were active above background in newborn rat retina or brain cells, the two non-lens cell types used, confirming the tissue-specificity of the γD promoter (Figure 5). Based on our previous results using chicken cells, we suspected that one reason for the tissue-specificity of the promoter might be the presence of a non-lens silencer element located between −85 and −67. To test this hypothesis, pBLCAT2 constructs containing single or multiple copies of a double stranded oligonucleotide spanning this region were transfected in rat lens cells and in several non-lens rat tissues. The expression of these constructs was considerably lower than that of the parental pBLCAT2 plasmid when tested in primary cultures of rat brain, retina or liver cells. In contrast, the same constructs transfected into rat lens cells did not result in a decrease in CAT activity as compared to the activity of the pBLCAT2 construct (Figure 7). This strongly suggests that the region between −85 to −67 of

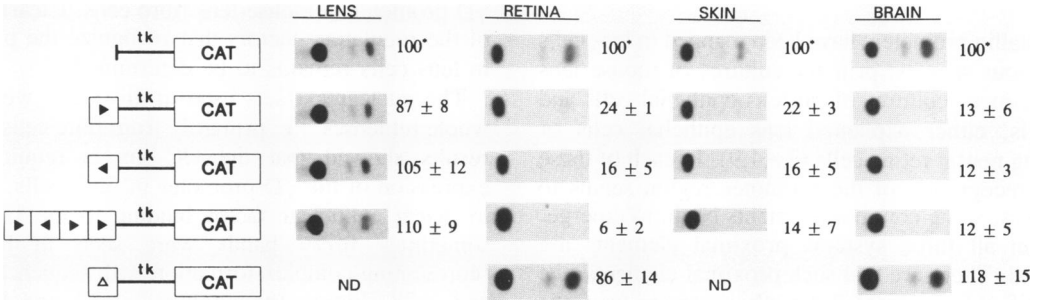

Figure 7. The region between −85 and −67 displays a strong silencer effect in non-lens tissues. pBLCAT2γ constructs containing one or multiple copies of an oligonucleotide spanning the γD promoter region from −85 to −67 were transfected into primary cultures of newborn rat lens, retina, brain or liver cells. Arrowheads indicate the orientation and number of the inserted oligonucleotide. In the construct pBLCAT2γΔ nucleotides −79 to −67 of the oligonucleotide are deleted by nuclease treatment. ND = not determined. Activities are given relative to that obtained from pBLCAT2 (100*).

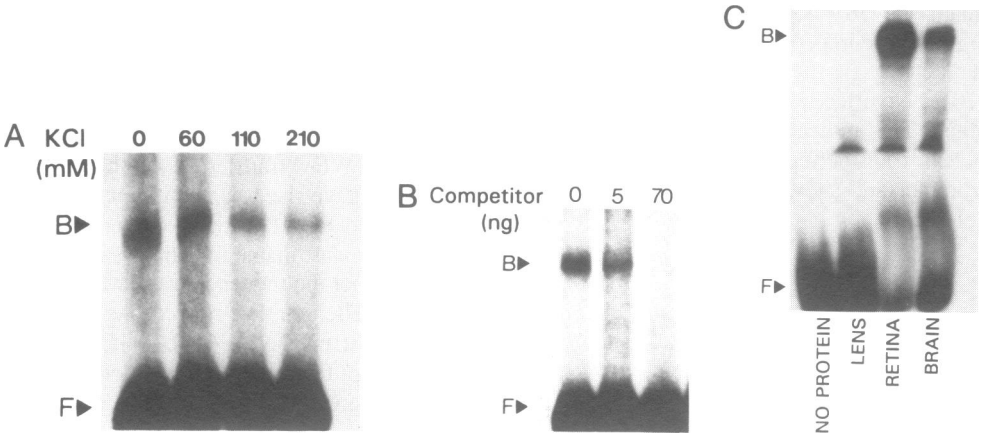

Figure 8. Complex formation of the silencing sequences (−85 to −67) with rat tissue nuclear extracts. **(A)** Salt resistance of the complex. Radiolabelled γD promoter silencing region was incubated with newborn rat brain nuclear extract at increasing salt concentration as indicated. Bandshift assay were performed as described in Materials and Methods except that a 4% polyacrylamide gel was used. Free DNA (F) and complexes (B) are indicated. **(B)** Saturation of the complex. Complex formation of the silencer region with newborn rat brain nuclear extract was performed in the presence of increasing amounts of non-labelled −85/−67 γD promoter fragment. The complex formation was measured by analysis on a 4% polyacrylamide gel. **(C)** Tissue-specificity of the complex. A radiolabelled restriction fragments containing the γD promoter silencer region was incubated with nuclear extracts either from newborn rat lens, retina or brain cells. Bandshift assays were performed as in A or B except that a 6% polyacrylamide gel was used.

the γD-crystallin promoter contains a silencer, active in non-lens cells but not in lens cells. Deletion of part (−79 to −67) of this sequence by nuclease treatment completely abolished the silencing effect in rat brain and retina cells (Figure 7). Note, however, that deletion of the silencing region does not activate the 'minimal' γD promoter in non-lens cells (Figure 5), suggesting that a tissue-specific positive regulatory factor is required for expression as well. This suggestion is in agreement with the lack of detectable gamma-box binding activity in retina or brain cells.

To test whether the silencing effect requires an interaction with a nuclear factor, a competition experiment was again performed. Co-transfection with a five-fold excess of the silencer region in pUC with the pBLCAT2γ-silencer construct had no effect on the activity of the promoter. However, co-transfection with 0.5 μg of a double stranded oligonucleotide containing the silencer sequence did abolish the silencing effect (data not shown). This effect must therefore be mediated by a DNA binding factor. Indeed, nuclear extracts of brain or retina cells, but not lens cells, contain a factor forming a salt-resistant, saturable complex with this region (Figure 8).

DISCUSSION

The rodent γ-crystallin promoters have been mapped in lens cells isolated from various species: primary cultures of mouse lens epithelial cells, primary cultures of rat lens epithelial cells and chicken lens cells, either explanted lens epithelial cells or transdifferentiating neural retina cells (2−4,9). In each of these cell systems the recognition of the promoter region seems to differ. Nevertheless, some common elements begin to emerge. For expression in all three systems proximal elements are absolutely required. There are two such proximal elements, the gamma-box identified here, located directly upstream from the TATA box, and the GC-box, located around −73. In primary cultures of mouse lens cells, deletion of the GC-box lowered expression by 50% (2). Curiously, as shown here, the GC-box does not seem to be required in primary cultures of rat lens epithelial cells. It seems very unlikely that the difference in recognition of the GC-box between rat and mouse lens cells represents a species-specific difference. Rather, we suggest that it correlates with the differentiation state of the cell. As shown previously (2), the mouse cells differentiate rapidly, express the endogenous crystallins abundantly and form copious lentoid bodies. In contrast, as shown here, the rat cells retain their epithelial properties. Apparently in lens epithelial cells, as exemplified by the rat cell system, a single element, the gamma-box, suffices to drive the γ-crystallin promoter, while in fibre cells, i.e. in the mouse primary cultures, a second element, the GC-box, is required as well. An obvious hypothesis would be that the gamma-box serves as a basal lens specific element, while the GC-box would augment the rate of transcription in the fibre cells. However, at present our data offer no support for this hypothesis. The transfected γD promoter constructs are as active—compared to either the tk or the SV40 promoter—in (rat) lens epithelial cells as in (mouse) lens fibre cells, hence the 'basal' rate of transcription would appear to be as high as the 'augmented' rate of transcription. In addition, the GC-box is unable to act as an enhancer in mouse lens cells (2). Finally, deletion up to −73, which leaves only the minimal promoter and should not affect the basal rate of transcription, completely inactivates the γD promoter in mouse lens fibre cells. Clearly, the interaction of the regulatory factors that recognize the proximal elements in lens cells remains to be determined.

The nuclear extracts used in this study were obtained from whole rat lenses, i.e. primarily from fibre cells. As our previous results showed that the GC-box is required for maximal expression of the γD-promoter in fibre cells, we had expected to detect a nuclear factor binding to the GC-box. Although sometimes fuzzy bands were seen in bandshift assays, representing complex formation with sequences between −200 and −78 (Figure 1c, compare lane 1 and lane 2), no clear interactions could be demonstrated. Similarly, in a study of binding factors in chicken lenses (9), no factor for the GC-box was detected although in chicken lens cells the GC-box is required for maximal expression of rodent γ-crystallin promoters. The lack of a detectable GC-box factor might be explained if it is

unstable or if its concentration is too low to be detected under our conditions. We favour the latter alternative, since our recent results show that transcription of crystallin genes is restricted to intermediate fibre cell differentiation stages (24), which form only a minor fraction of the fibre cells found in the total lens.

Although chicken lenses do not contain the homologs of the rodent γA−γF crystallins, the gamma-box and the GC-box are recognized by chicken lens cells. The mode of action of the GC-box differs from that in mouse cells, as the GC-box can act as a lens specific enhancer in chicken cells (2). A nuclear factor that binds the gamma-box is found in chicken lens cells as well (9). This result highlights the evolutionary conservation of the gamma-box factor. As this factor may be confined to lens cells and appears to be conserved in vertebrates, this factor could be the prime determinant of lens-specific expression.

The GC-box has a dual role: it is required for expression in lens fibre cells but it also acts as a silencer element in non-lens cells. The functional relevance of this finding for the silencing of γ-crystallin genes *in vivo* is moot, as methylation of the chromosomal DNA presumably already prevents activation of these genes (25). There is, however, a strong sequence resemblance between this γ-crystallin element and a region within the βB1-crystallin promoter (26). Perhaps the silencer functions *in vivo* to control the activity of the β-crystallin genes.

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