Lens-specific activity of the chicken δ1-crystallin enhancer in the mouse

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ABSTRACT A lens-specific enhancer was identified in the third intron of the chicken δ1-crystallin gene by analysis based on transient transfection of primary-cultured cells. To assess the significance of this enhancer's activity in embryonic lens cells during development, tkCAT gene carrying the enhancer was introduced into mouse embryos utilizing ES (embryonic stem) cell-mediated gene transfer. In the undifferentiated culture condition, ES lines with enhancer-carrying tkCAT did not express any significant level of CAT (chloramphenicol acetyltransferase). However, when the ES cells were injected into a blastocyst and allowed to differentiate into various somatic cells of an embryo, CAT expression was observed exclusively in lens, and the expression was dependent upon the δ1-crystallin enhancer. We concluded that the δ1-crystallin enhancer alone is sufficient for eliciting lens-specific gene expression in developing mouse embryos and that the mechanism of lens-specific regulation effected by the δ1-crystallin enhancer is conserved between the chicken and the mouse.

KEY WORDS: *δ-crystallin, enhancer, ES cells, mouse chimera*

Introduction

The δ1-crystallin gene is the first lens-specific gene to be expressed in avians (Piatigorsky, 1981). Therefore, the molecular mechanisms which regulate expression of this gene are highly relevant to the process of lens cell differentiation.

Lens specificity of δ1-crystallin expression is determined by the enhancer in the third intron of the gene, as assayed by transfection of primary chicken cells (Hayashi *et al.*, 1987). Nuclear factors which interact with the δ1-crystallin enhancer and participate in eliciting lens-specific enhancer activity have been identified in chicken embryos (Funahashi *et al.*, 1991, 1993; Kamachi and Kondoh, 1993).

Although the δ1-crystallin gene is absent in mammals, the cloned chicken gene is regulated in a lens-specific manner in mouse cells, including cells in primary culture (Kondoh *et al.*, 1983), mouse embryos (Takahashi *et al.*, 1988) and adult mice (Kondoh *et al.*, 1987), suggesting that the basic mechanism of lens-specific regulation is conserved between the chicken and the mouse. In support of this notion, it has been shown that a mammalian γF-crystallin gene, which is missing from the chicken, is also regulated correctly in the chicken lens cells (Lok *et al.*, 1985).

To further evaluate developmental roles of the lens nuclear factors identified in the chicken system, the genetic system of the mouse provides an invaluable tool, especially because of the availability of targeted mutagenesis taking advantage of ES cells (Thomas and Capecchi, 1987). It has not yet been established, however, whether the chicken δ1-crystallin enhancer is the target of the lens-specific regulation in the mouse.

In this study, we introduced the chicken δ1-crystallin enhancer combined with a CAT-coding gene into developing mice by producing chimeric embryos with DNA-transfected ES cells. We found that the chicken δ1-crystallin enhancer activates the CAT gene expression in a lens-specific manner in the mouse.

Results and Discussion

Transformant ES CP1 lines carrying tkCAT with the δ1-crystallin enhancer

CP1 cells were transfected with tkCAT plasmid with or without the δ1-crystallin enhancer and were selected for G418 resistance utilizing the tkneoB activity of the same plasmid. No enhancer element other that the δ1-crystallin enhancer was present on the transfected plasmids so that the effect of the δ1-crystallin enhancer was uniquely assessed. Five G418-resistant clones were obtained from 10^6 transfected CP1 cells when tkCAT carried the enhancer, whereas 30 clones were obtained from the same number of cells when no enhancer was on the plasmid.

Southern blot analysis of the transformant clones indicated that those carrying the δ1-crystallin enhancer tended to have higher

Abbreviations used in this paper: ES, embryonic stem; CAT, chloramphenicol acetyltransferase; GPI, glucose-6-phosphate isomerase.

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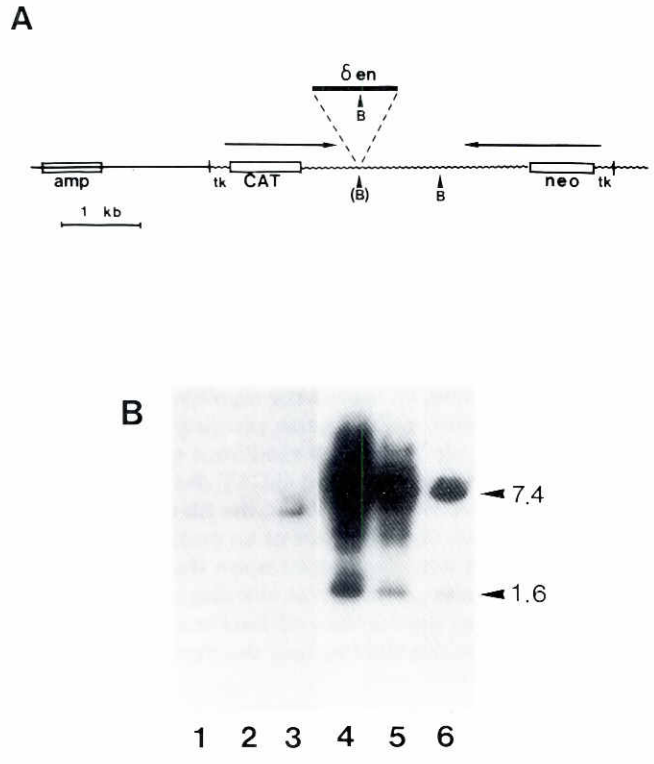

Fig. 1. tkCAT-tkneoB plasmids introduced into CP1. (A) *Organization of the plasmid. tk, Herpes virus thymidine kinase promoter; ~, SV40 intron-termination signal region; CAT and neo, bacterial antibiotic-resistant genes; δen, δ1-crystallin enhancer (Hayashi et al., 1987); —, vector pBR322 sequence. BamHI restriction sites are indicated by B with arrowhead. The site indicated by (B) was lost when the δen sequence was inserted.* **(B)** *Southern blot of the transformant lines. BamHI digests of the DNA of the lines, (1) O-4, (2) O-5, (3) O-7, (4) δ-6, (5) δ-16, are compared with (6), a BamHI digest of the tkCAT-tkneoB plasmid carrying the δ1-crystallin enhancer, showing characteristic 7.4 and 1.6 kb bands.* **Lanes 1 to 3** *were exposed four times longer than* **4 to 6**. *The band intensity of the reference plasmid DNA in* **lane 6** *corresponded to 10 copies per diploid genome DNA in other lanes.*

multiplicity of the exogenous DNA copies than those without the enhancer (Fig. 1B). The lower yield of G418-resistant transformants and a higher multiplicity of the transfected DNA sequence found in the transformants when the δ1-crystallin enhancer was in the plasmid are explained if one assumes a repressing effect of the δ1-crystallin enhancer in ES cells, analogous to Moloney leukemia virus enhancer (Gorman *et al.*, 1985). The enhancer probably lowered expression of the tkneoB gene on the same plasmid in ES cells, and as a consequence only such transformants that carried a high enough copy number survived the G418 selection.

CAT expression in undifferentiated ES cells

Several transformant ES lines (tkCAT-ES lines) in the undifferentiated state were tested for CAT expression (Fig. 2). Although some of those without the enhancer exhibited low but appreciable CAT activity, none of the tkCAT-ES lines carrying the δ1-crystallin enhancer expressed CAT activity. This is also explained by the negative effect of the δ1-crystallin enhancer on the undifferentiated ES cells, which repressed not only the tkneoB but tkCAT gene expression.

CAT expression in chimeric mouse embryos

Two tkCAT-ES lines carrying the δ1-crystallin enhancer (δ–6 and δ–16) as well as a tkCAT line without the enhancer (O-7) were used to produce chimeric mouse embryos. These cells were injected into blastocysts derived from the mating between C57/B6 and C3H mice, and transferred to uteri of pseudopregnant Balb/c mice. The ES line CP1 was derived from 129 mouse strain and hence its GPI (glucose-6-phosphate isomerase) was of *a*-isotype, whereas the recipient mice had GPI-*b* isotype. Therefore, the chimerism of the embryos developed from ES-injected blastocysts was assessed by the isozyme analysis. The embryos were recovered at stages around 13 days (Theiler, 1989), and tissue extracts of brain, limb, heart, lung and liver were analyzed for GPI isotypes. Chimeric embryos displaying a wide tissue distribution of ES-derived cells (i.e., presence of GPI-*a* isotype in every tissue examined) were identified. Three examples, δ–6/7-2, δ–16/2-4 and O-7/8-3, are shown in Fig. 3A. Since ES-derived cells appeared to be distributed ubiquitously, lenses of these embryos were also expected to contain ES-derived cells, although these lenses were assessed only for their CAT activity (see below) for their small amount of the tissues.

Tissues of the same chimeric embryos, δ–6/7-2, δ–16/2-4 and O-7/8-3 were examined for CAT expression (Fig. 3B). In the chimeras with tkCAT with the δ1-crystallin enhancer (δ–6/7-2 and δ–16/2-4), a significant level of CAT activity was present in the lens, but no activity in other tissues was observed. In the chimeras of the line O-7 which had tkCAT gene without δ-crystallin enhancer, CAT expression was negative in all tissues, although the same ES cell line expressed an appreciable level of CAT activity in the undifferentiated state (Fig. 2A). Other chimeras with lines O-4 and

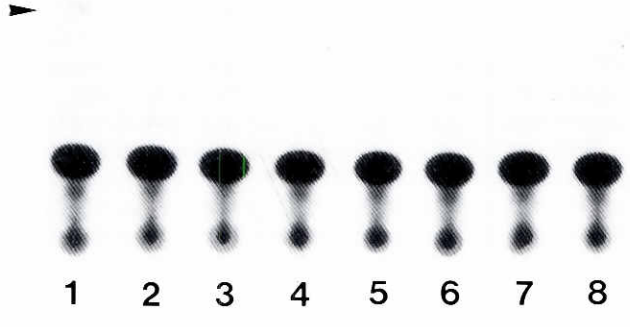

Fig. 2. CAT expression of the transformant ES cells in the undifferentiated state. *Cell extract from ca. 2x10⁶ were used for standard CAT assay (Hayashi et al., 1987). Transformant ES lines: (1) O-4, (2) O-5 and (3) O-7 were tkCAT-tkneoB lines without the δ1-crystallin enhancer, while (4) δ-1, (5) δ-5, (6) δ-6, (7) δ-16 and (8) δ-27 were those with the δ1-crystallin enhancer. The arrowhead indicates the position of 3-acetylated chloramphenicol, which was detectable in extract of the lines O-4 and O-7.*

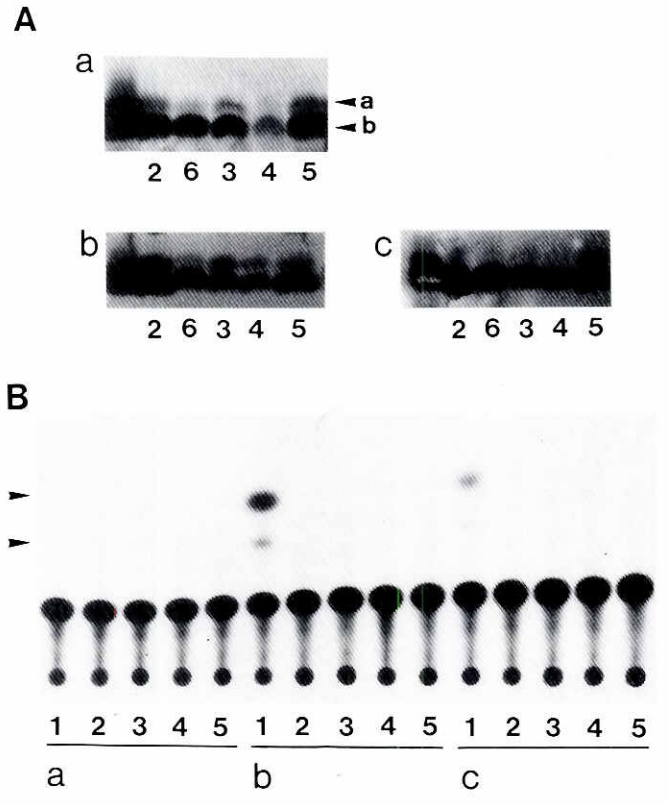

Fig. 3. CAT expression in chimeric embryos displaying a wide tissue distribution of ES-derived cells (A) *GPI analysis to demonstrate the distribution of the ES-derived cells. (a) Chimera O-7/8-3, (b) chimera δ-6/7-2, (c) chimera δ-16/2-4. Tissues: (2) brain; (6) limbs; (3) heart; (4) lung; (5) liver; unmarked, tissue extract from GPI-α/ß heterozygous mouse as reference. a and b with arrowheads indicate positions of GPI-α and -ß, respectively.* **(B)** *CAT expression in the chimeric tissues. Chimeras were the same as in* **(A)**. *Tissues were: (1) lens (1 μg protein); (2) brain (100 μg protein); (3) heart (40 μg protein); (4) lung (10 μg protein) and (5) liver (100 μg protein). The positions of 1- and 3-acetylated chloramphenicol are indicated by arrowheads.*

O-7 expressed no CAT activity in the lens (data not shown). Thus, the chicken δ-crystallin enhancer elicited lens-specific expression of the tkCAT gene in mouse embryos.

Conclusion

The results described above demonstrated that the tkCAT gene is exclusively expressed in the lens tissue of the mouse embryo when the gene carries the chicken δ1-crystallin enhancer, but is not expressed in the absence of the enhancer. This indicates that the mouse and the chicken share a conserved lens-specific regulatory mechanism which is effective on the δ-crystallin enhancer. The region of the enhancer responsible for eliciting lens-specific transcriptional activation has been defined (Goto *et al.*, 1990; Funahashi *et al.*, 1991; Kamachi and Kondoh, 1993), and transcription factors interacting with the region have been identified in the chicken (Funahashi *et al.*, 1991, 1993; Kamachi and Kondoh, 1993). It is likely that these factors are conserved between the chicken and mouse, and underlie the conserved mechanism of regulation of the δ-crystallin enhancer. It will be interesting to determine which of the lens-specific genes of the mouse rely on the same regulatory mechanism as the δ1-crystallin enhancer.

Materials and Methods

Construction of tkCAT-tkneoB with δ1-crystallin enhancer

Plasmids were constructed which carry the CAT-coding and G418-resistance genes but without any enhancer elements other than the δ1-crystallin gene (Fig. 1A). The tkCAT plasmids with or without the δ1-crystallin enhancer (Hayashi *et al.*, 1987) were cleaved at the ApaI site downstream of the CAT gene, blunt-ended by T4 DNA polymerase, and the blunt-ended HindIII-XhoI fragment of pSTneoB carrying the tkneoB sequence (Katoh *et al.*, 1987) was inserted in the orientation of transcription opposite to CAT transcription.

Transfection of ES CP1

ES CP1 cells (Bradley *et al.*, 1984) grown on neo-resistant feeder cells (Takahashi *et al.*, 1988) were transfected with plasmid DNA by a conventional calcium-phosphate method (Kondoh *et al.*, 1984). Transformants were selected by G418 at 200 μg/ml, and resistant colonies were obtained after 8 to 12 days.

Other methods

Southern blotting (Kondoh *et al.*, 1984), chimera mouse production (Takahashi *et al.*, 1988), GPI analysis (Hanaoka *et al.*, 1986) and CAT assay (Hayashi *et al.*, 1987) were performed according to published procedures.

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