Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 4681–4685, May 1995
Biochemistry

Pax-6 and lens-specific transcription of the chicken δ1-crystallin gene

ALEŠ CVEKL, CHRISTINA M. SAX, XUAN LI, JOAN B. MCDERMOTT, AND JORAM PIATIGORSKY*

Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Building 6, Room 201, Bethesda, MD 20892-2730

Communicated by J. Edward Rall, National Institute of Diabetes and Digestive Diseases, Bethesda, MD, January 3, 1995 (received for review November 7, 1994)

ABSTRACT **The abundance of δ-crystallin in the chicken eye lens provides an advantageous marker for tissue-specific gene expression during cellular differentiation. The lens-specific expression of the δ1-crystallin gene is governed by an enhancer in the third intron, which binds a positive (δEF2) and negative (δEF1) factor in its core region. Here we show by DNase I footprinting, electrophoretic mobility-shift assays, and cotransfection experiments with the δ1-promoter/enhancer fused to the chloramphenicol acetyltransferase reporter gene that the δ1-crystallin enhancer has two adjacent functional Pax-6 binding sites. We also demonstrate by DNase I footprinting that the δEF1 site can bind the transcription factor USF, raising the possibility that USF may cooperate with Pax-6 in activation of the chicken δ1- and αA-crystallin genes. These data, coupled with our recent demonstration that Pax-6 activates the αA-crystallin gene, suggest that Pax-6 may have been used extensively throughout evolution to recruit and express crystallin genes in the lens.**

Crystallins comprise 80–90% of the soluble protein in the ocular lens (1, 2). In the chicken, δ-crystallin constitutes 60–70% of the soluble protein of the embryonic lens, and the α- and β-crystallins make up the rest (3–5). Crystallin synthesis is temporally and spatially regulated in the developing chicken lens, with δ-crystallin appearing first in the presumptive lens ectoderm during placode formation (6) followed by the β-crystallins in the early lens vesicle and finally the α-crystallins in the elongating primary fiber cells (7). Transfection and transgenic mouse experiments have indicated that transcriptional control plays a major role in regulated expression of the crystallin genes (8, 9).

In addition to its refractive role in the lens, δ-crystallin is argininosuccinate lyase (10). There are two extremely similar, tandemly linked δ-crystallin genes (δ1 and δ2) in the chicken, encoding proteins with 91% sequence identity (11, 12). Both genes are expressed to limited extents in numerous non-lens tissues (13–15). Only the δ2-crystallin polypeptide has enzymatic activity (16, 17). The δ1-crystallin polypeptide is specialized for its crystallin function and is ≈100 times more prevalent in the embryonic chicken lens than the δ2-crystallin polypeptide (13–15, 18). An enhancer in the third intron is responsible for the high expression of the δ1-crystallin gene in the lens (19).

It is widely recognized that the molecular mechanisms governing embryonic patterning and tissue differentiation are driven by restricted expression of transcription factors, which activate specific genes and generate a diverse array of cell types (20). Of particular interest with respect to the eye is Pax-6 (21–26), a paired domain (PD)- and homeodomain-containing protein. Mutations of Pax-6 result in distinct eye abnormalities including small eye in mouse (21), aniridia (24, 27, 28) and Peters anomaly in human (29), and eyeless in *Drosophila* (30). Recently, we have found that Pax-6 is a critical transcription factor involved in expression of chicken (31) and mouse (32) αA-crystallin genes in the lens. In addition, it is also demonstrated (33) that the regulatory sequence directing expression of the guinea pig ζ-crystallin gene utilizes Pax-6.

Here we report the presence of two functional Pax-6 binding sites located 50 bp upstream from the δEF2 and δEF1 cis control elements in the B4 (core) region of the δ1-crystallin enhancer (34). We also provide DNase I footprinting evidence that USF binds to the δ1 enhancer at a site overlapping the previously reported δEF1 repressor binding site (34). Our results fit with the idea that Pax-6 is a critical transcription factor for the high expression of many different crystallin genes in the lens.

MATERIALS AND METHODS

Cell Culture, Transfections, and Expression of Pax-6. Embryonic chicken primary lens epithelial cells (PLEs) and fibroblasts (CEFs) were cultured as described (31). Transfections, cotransfections with pKW10-Pax-6 (35), and chloramphenicol acetyltransferase (CAT) and β-galactosidase assays were conducted and evaluated as described elsewhere (31).

Oligonucleotides and Plasmid Constructions. The following oligonucleotides were used: IIa (+1827 to +1857; +1 indicates transcription initiation site), 5′-AGTAGAAGACAATGCACAATATTGTATAGGG-3′; IIb (+1803 to +1835), 5′-CCTTTGTGTCTGGCTGCCTGAGTTAGTAGAAGA-3′; Pax-6 PD binding site (36), 5′-GGATGCAATTTCACGCATGAGTGCCTCGAGGGATC-3′; oligonucleotide E (31). pδ1 was prepared by subcloning the −51 to +59 δ1-crystallin promoter fragment into *Pst* I/*Hin*dIII-digested p8-CAT (37). To construct pδ1B5, the B5 enhancer region (+1922 to +2046) of the δ1-crystallin gene was subcloned as a *Nco* I/*Pst* I fragment into pδ1. To construct pδ1WT, the B3 and B4 regions (+1706 to +1922) were subcloned as *Sal* I/*Nco* I fragments into pδ1B5 (Fig. 1*B*). Deletions in pδ1WT were generated by the PCR procedure, oligonucleotides containing the desired mutation, and Vent DNA polymerase (New England Biolabs). All constructs were verified by direct sequencing. Sequences of the δ1-crystallin gene are available in the GenBank data base under accession no. M10806.

Nuclear Extracts, Proteins, Electrophoretic Mobility-Shift Assay (EMSA), and DNase I Footprinting. Nuclear extracts from 14-day-old embryonic chicken lenses were prepared according to Shapiro *et al.* (38). For footprinting experiments, DNA-binding proteins from lens nuclear extract were partially purified on a heparin-Ultrogel A4R column and eluted with 400 mM KCl as described elsewhere (39). Purified human USF was a gift of Emery Bresnick (National Institutes of Health) (40), purification of Pax-6 PD (128 amino acids) expressed in *Escherichia coli* will be described elsewhere, and anti-Pax-6 antiserum 11 was a gift of Simon Saule (Institut Pasteur, Lille, France) (41). DNase I footprinting and EMSA were per-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PLE, primary lens epithelial cell; CEF, chicken embryonic fibroblast; CAT, chloramphenicol acetyltransferase; PD, paired domain; EMSA, electrophoretic mobility-shift assay.

*To whom reprint requests should be addressed.

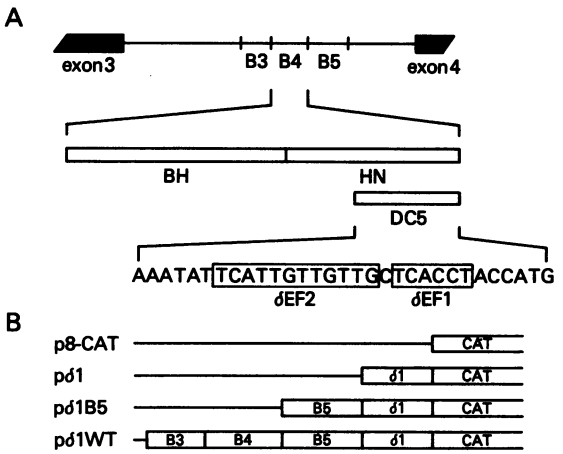

FIG. 1. Schematic representation of δ1-crystallin enhancer and structure of CAT reporter plasmids. (*A*) Full enhancer activity was reconstituted from regions B3, B4 (core), and B5 (19). Two protein binding sites, δEF2 and δEF1, are boxed in fragment DC5 (34). The sequence of the BH fragment, which includes the Pax-6 binding sites, is shown in Fig. 2*C*. (*B*) Structure of plasmids prepared from p8-CAT. δ1, Homologous promoter fragment −51 to +59; enhancer regions B3, B4, and B5 are from *A*.

formed as described (31, 39). The amounts of nuclear extracts or purified proteins were determined by prior titrations and are given in the figure legends.

RESULTS

DNase I Footprinting of the B4 Region of the δ1-Crystallin Enhancer. The third intron enhancer of the chicken δ1-crystallin gene is shown schematically in Fig. 1*A*. The B4 region provides core enhancer activity in the lens, while regions B3 and B5 are important for full enhancer activity (19). Two protein binding sites, δEF1 and δEF2, have been identified within DC5, a subregion of B4 (34). The reconstitution of lens-specific enhancer activity in transfected lens cells, however, required multiple copies of DC5 (34). This suggested to us that additional cis-acting elements may be located in the B4 core enhancer region. We thus performed DNase I footprinting of the chicken δ1-crystallin sequence +1706 to +1922, including enhancer regions B3 and B4, using partially purified nuclear extracts prepared from 14-day-old embryonic chicken lenses. Three footprinted regions (I, II, and III) were detected on the upper (sense) strand (Fig. 2*A*), while two footprinted regions (I and II) were detected on the lower (antisense) strand (Fig. 2*B*). Region I is located within B3 and region II is within B4 (core); region III (footprinted weakly) maps to the previously identified binding sites δEF2 and δEF1 (34). The reason that the region III footprint is weak may be attributed to the use of binding conditions different than those reported elsewhere for EMSAs (34).

We considered Pax-6 as a candidate binding protein to region II since the footprinted sequences matched previously identified Pax-6 binding sites (31, 32). Both the 128-amino acid Pax-6 PD and an extract prepared from COP-8 fibroblasts transfected with a Pax-6 cDNA expression vector (see *Materials and Methods*) generated a region II footprint similar to that observed with chicken lens extract (Fig. 2*A*, lanes 8–10; Fig. 2*B*, lanes 7–9). Unexpectedly, Pax-6 PD also interacted with region III in the DNase I footprinting experiments. Protein binding to regions IIa and IIb were also investigated by EMSAs with synthetic oligonucleotides spanning these sequences. A single major specific complex, δP6, was detected

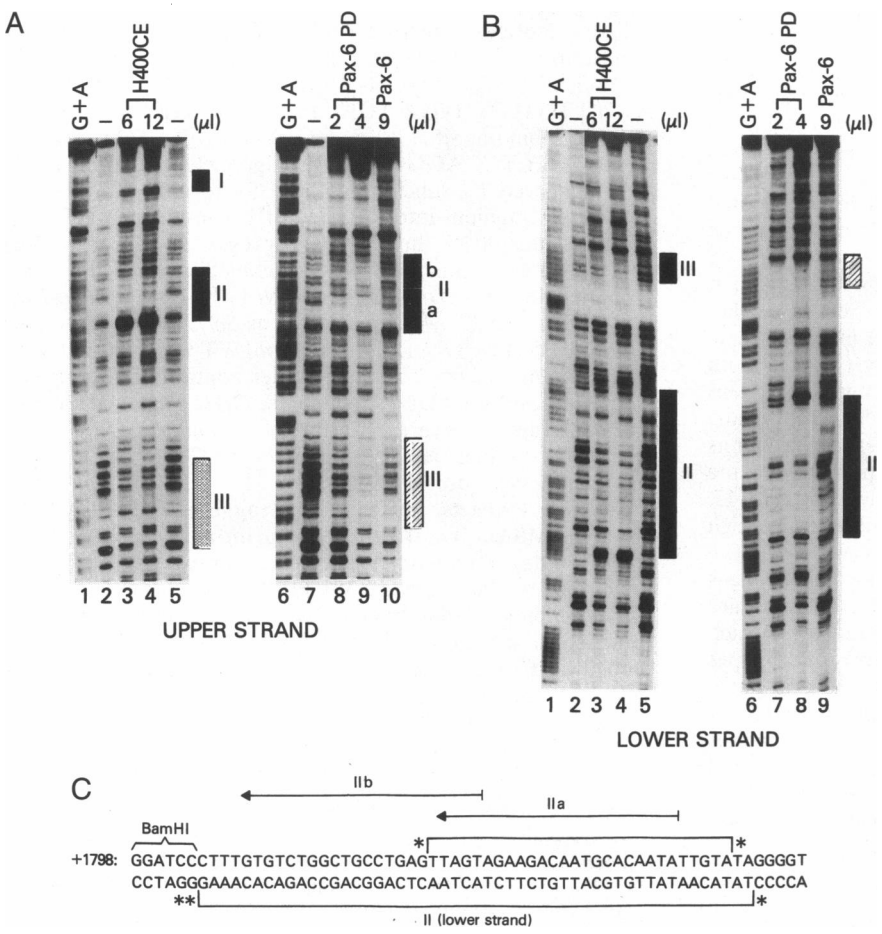

FIG. 2. DNase I footprinting of the chicken δ1-crystallin enhancer regions B3 and B4 (core). Footprinting was performed with the indicated volumes of partially purified embryonic chicken lens nuclear extract (H400CE, 0.6 mg/ml), Pax-6 PD (0.67 mg/ml), or COP-8 fibroblast extract containing Pax-6 (Pax-6 WT, 2.2 mg/ml) as described. Lanes G+A, Maxam–Gilbert G+A reactions. (*A*) Upper (sense) strand footprint. Footprinted regions are shown as labeled boxes (I, II, or III). Hatched box represents a relatively weak footprint. Region II footprinted with the Pax-6-containing nuclear extract is shown as boxes IIa and IIb. (*B*) Lower (antisense) strand footprint is labeled according to *A*. (*C*) Nucleotide sequence of the Pax-6 binding sites; these are in fragment BH (see Fig. 1*A*). *, DNase I hypersensitive site.

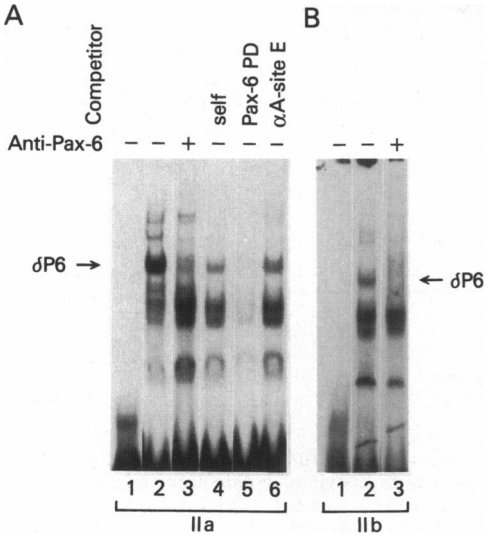

FIG. 3. EMSA analysis of proteins binding to region II. δP6 is the major specific complex detected on probes IIa (*A*) and IIb (*B*). Anti-Pax-6 is antiserum 11 against PD of Pax-6 (41). Oligonucleotides Pax-6 PD and αA-site E (50-fold molar excess over the probe) are described in *Materials and Methods.*

with both the IIa (Fig. 3*A*) and IIb (Fig. 3*B*) oligonucleotides. An antiserum against quail Pax-6 PD (41) inhibited formation of complex δP6 (lanes 3). In addition, competition with oligonucleotides Pax-6 PD (36) (lane 5) or site E (31) of the chicken αA-crystallin gene (lane 6), both known to bind Pax-6, reduced the formation of complex δP6. We conclude from these data that the B4 (core) region of the δ1 enhancer has binding sites for Pax-6 (Fig. 2*C*).

Transfections. We next investigated whether the Pax-6/region II protein binding site has a functional role in enhancer activity by performing transfection experiments. A series of homologous promoter/enhancer constructs fused to the CAT reporter gene (Fig. 1*B*) were prepared and transfected into chicken PLEs and CEFs. The complete enhancer B3-5 fused to the δ1 promoter (pδ1WT) activated the δ1 promoter 4- to 5-fold in transfected PLEs but not in transfected CEFs (data not shown). Deletion of the Pax-6 binding site IIa (+1830 to +1854) in pδ1(ΔP6) resulted in a 40% decrease in promoter activity, and deletion of complete site II (+1808 to +1854) in pδ1(ΔΔP6) resulted in a 50% decrease of promoter activity relative to pδ1WT (data not shown). The possibility that Pax-6 can activate the chicken δ1-crystallin enhancer/promoter was examined by cotransfecting CEFs with pδ1WT and a mouse Pax-6 cDNA expression vector (pKW10-Pax-6) (35). pKW10-Pax-6 stimulated pδ1WT activity in the cotransfected CEFs (Fig. 4); the parental vector (pKW10) lacking Pax-6 coding sequences had no effect on pδ1WT activity (data not shown). Increasing amounts of pKW10-Pax-6 (0.5–3 μg of plasmid) gave concentration-dependent curves similar to that found for transactivation of chicken αA-crystallin promoter (31). Moreover, deletion of the Pax-6 binding site at region IIa (pδ1ΔP6) or of both Pax-6 binding sites of region II (pδ1ΔΔP6) from pδ1WT reduced most of the transactivating effect of pKW10-Pax-6 in cotransfected CEFs. A weak Pax-6 binding site through its paired domain at footprinted region III of the δ1-crystallin enhancer could be responsible for the limited activation of pδ1ΔΔP6 that occurred in these experiments.

USF Binds to the δEF1 Site. Previous experiments have suggested that a positively acting element overlaps the negatively acting δEF1 site of the δ1-crystallin enhancer (34, 42). δEF1 is a zinc finger homeodomain protein (43). We have noted that the sequence 5′-GCTCACCTACCA-3′, which includes the δEF1 site (underlined), shows some similarity with the binding site for the helix–loop–helix transcription factor USF (5′-GGG/CCACG/ATGAC-3′) (44). USF is present in the embryonic chicken lens nuclear extract and binds to two sites in the chicken αA-crystallin promoter (31). Thus, we performed DNase I footprinting experiments with the +1706

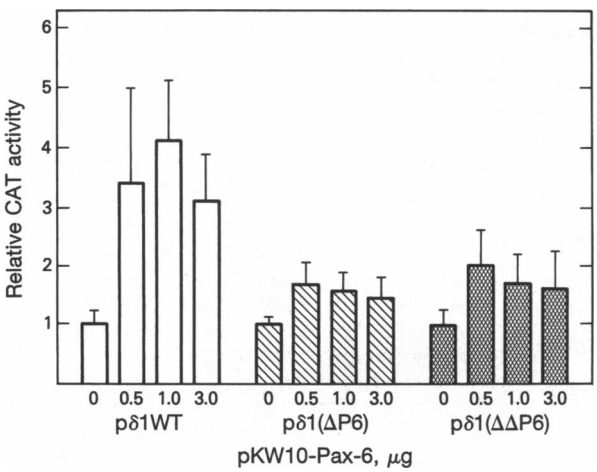

FIG. 4. Cotransfections of CEFs with Pax-6 and different δ1-promoter/enhancer constructs. CEFs were cotransfected with pδ1WT, pδ1ΔP6, or pδ1ΔΔP6 and increasing amounts of pKW10-Pax-6 (35), a mouse Pax-6 cDNA expression vector. Data are expressed as ratio of normalized CAT activity (average + SD) in the presence and absence of pKW10-Pax-6.

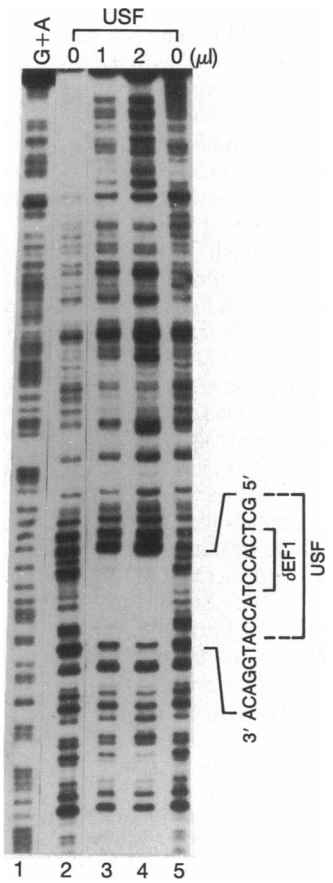

FIG. 5. DNase I footprinting of chicken δ1-crystallin enhancer regions B3 and B4 with purified human USF (300 ng/μl; 90% purity; see ref. 40). Experimental conditions were described elsewhere (31). Protected region is bracketed. USF and δEF1 recognition sequences are indicated.

to +1922 labeled δ1-crystallin enhancer fragment and purified human USF (40). The result showed that the δEF1 site was indeed protected from DNase I digestion after incubation with purified USF (Fig. 5).

DISCUSSION

In situ hybridization (22, 23, 25) and mutant phenotypes (21–24, 27–30) have implicated Pax-6 in eye development. Pax-6 has been especially associated with early developmental and inductive events of eye formation, when crystallin synthesis begins (see refs. 45–47). We have recently shown that Pax-6 binds to and activates the chicken (31) and mouse (32) αA-crystallin promoters. The present investigation provides transfection, immunological, and DNA-binding evidence that Pax-6 can also activate the δ1-crystallin enhancer (19), implicating Pax-6 in the tissue-specific expression of at least two entirely different crystallin genes in the developing chicken lens. Activation of δ1-crystallin gene expression by Pax-6 fits well with the developmental timing for expression of the δ-crystallin (6) and Pax-6 (25) genes in the chicken embryo. It is thus likely that Pax-6 contributes to the high lens expression of the chicken δ1-crystallin transgene in transgenic mice (48, 49) even though the mouse lens does not have δ1-crystallin (see refs. 1 and 8). It is also possible that Pax-6 is responsible for activating the δ1-crystallin (48, 49) and δ2-crystallin (49, 50) enhancers in the brains of transgenic mice, where Pax-6 is normally expressed (23). The reason for the relatively low expression of the δ2-crystallin gene in the chicken lens (13–15, 18) remains problematic, since the δ2 gene contains a functional enhancer in intron 3 with a sequence similar to that in the δ1 gene (refs. 13 and 15; see also ref. 50). One possibility is that differential structural alterations within the chromatin, such as methylation (51, 52), make the δ2-crystallin gene inaccessible to Pax-6 or other transcription factors. Another possibility is that the activity of the δ2-crystallin gene is repressed by an as yet undiscovered silencer.

The studies of Kondoh and colleagues (34, 42, 43) have shown that lens-specific activity of the δ1-crystallin enhancer core (B4) can be recovered by octamerizing a 30-bp fragment (DC5; see Fig. 1), which can bind activators (δEF2a and δEF2b) in the lens and repressors (δEF2c, δEF2d, and δEF1) in numerous tissues, including the lens. It is important to note that octamers of the BH4 fragment (containing the two Pax-6 binding sites IIa and IIb) of the enhancer core (see Fig. 1) were not active in transfection tests (34, 53). This result is comparable to our transfection experiments with chicken αA-crystallin promoter constructs, where mutation of either site A (USF) or site B (CRE-like) eliminated promoter activity even if the Pax-6 binding sites (sites C and E) remained intact (31). Clearly, Pax-6 by itself is insufficient for activating either the chicken αA-crystallin promoter or the δ1-crystallin enhancer.

The existence of an activating site overlapping the δEF1 repressor site of the δ1-crystallin enhancer has been proposed (34). The DNase I footprinting experiments shown here suggest that USF may indeed be an activator of the δ1-crystallin enhancer. We have shown recently that USF also interacts with the chicken αA-crystallin promoter, where it serves as an activator in the lens and as a repressor in fibroblasts by binding to the composite element at site A and as a repressor in the lens by binding to site D (31). Thus USF, like Pax-6, appears to be a transcription factor that is used for at least two crystallin genes in the chicken lens. The activating roles of Pax-6, USF, and δEF2a and -b in the lens and the repressing roles of δEF1 and δEF2c and -d in fibroblasts are diagrammed in Fig. 6. Further data are required to determine whether changes in the relative amounts of USF, δEF1, and related proteins δEF2a–d modulate the spatial and temporal expression of the δ1-crystallin gene in the lens.

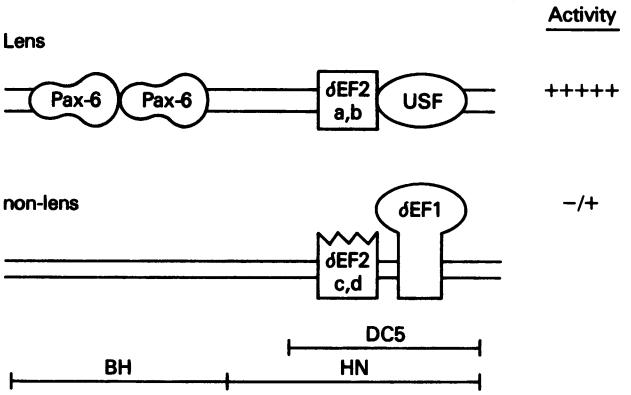

FIG. 6. Model for transcription factor interactions in the B4 (core) region of the chicken δ1-crystallin enhancer. In lens cells, Pax-6 binds to two adjacent sites, IIa and IIb. δEF2a and -b and USF are other activators. USF competes with repressor δEF1 (34, 42, 43). Contribution of multiple individual factors results in high expression (+++++) of δ1-crystallin in the lens. In non-lens cells, absence of any of the activators (Pax-6, USF, and δEF2a and -b) and/or the presence of repressors (δEF2c and -d and δEF1) results in low (−/+), if any, expression of the δ1-crystallin gene.

Finally, a list of regulatory sequences that have been associated with lens expression by direct functional studies indicates that Pax-6 may activate various crystallin genes (Fig. 7; refs. 31–33). We have also listed the neural cell adhesion molecule L1 gene in Fig. 7, since this regulatory sequence binds Pax-6 (54). The proposed Pax-6-binding site derived from this alignment is in general agreement with that for the 128-amino acid Pax-6 PD derived by the random oligonucleotide selection method (36). Other potential Pax-6 activating sites for lens expression implicated by sequence and/or binding studies include those regulating the mouse αB-crystallin (55), chicken βA3/A1-crystallin (J.B.M., A.C., and J.P., unpublished data), and squid SL11- and SL20-crystallin (56) genes. These data are consistent with the idea that Pax-6 may be a universal regulator of eye development (see refs. 26 and 29) and probably contributes to the high expression of many crystallin genes in the lenses of vertebrates and invertebrates. The utilization of Pax-6 for high expression of many different crystallin genes in the lens of multiple species may reflect the importance of selective pressures on transcription factors for the recruitment of crystallin genes and provide a common link unifying the diverse crystallin genes on the basis of their regulation rather than strictly on the phenotype of their encoded proteins (see refs. 9, 50, and 57).

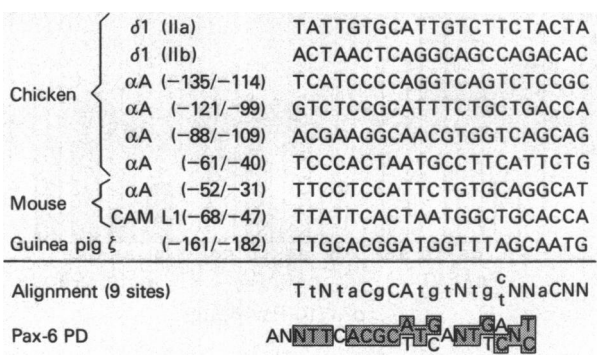

FIG. 7. Analysis of Pax-6 binding sites. Capital letters are used to indicate at least 67% conservation of each nucleotide, while lowercase letters indicate at least 44% conservation of each nucleotide. Consensus Pax-6 PD binding site is shown (36) and common nucleotides between alignment and Pax-6 consensus are shaded.

We are grateful to Drs. E. Bresnick, M. Busslinger, T. Czerny, and S. Saule for generous gifts of proteins, antisera, and clones; Dr. L. Segovia for help on computer analysis of DNA sequences; and M. Meyer for preparation of figures.

1. Wistow, G. J. & Piatigorsky, J. (1988) *Annu. Rev. Biochem.* **57,** 479–504.
2. Bloemendal, H. & deJong, W. W. (1991) *Prog. Nucleic Acids Res. Mol. Biol.* **41,** 259–277.
3. Genis-Galvez, J. M., Maisel, H. & Castro, J. (1968) *Exp. Eye Res.* **7,** 593–602.
4. Hejtmancik, J. F., Beebe, D. C., Ostrer, H. & Piatigorsky, J. (1985) *Dev. Biol.* **109,** 72–81.
5. Piatigorsky, J. (1981) *Differentiation* **19,** 134–153.
6. Shinohara, T. & Piatigorsky, J. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2808–2812.
7. Zwaan, J. & Ikeda, A. (1968) *Exp. Eye Res.* **7,** 301–311.
8. Piatigorsky, J. (1992) *J. Biol. Chem.* **267,** 4277–4280.
9. Piatigorsky, J. & Zelenka, P. S. (1992) *Adv. Dev. Biochem.* **1,** 211–256.
10. Piatigorsky, J., O'Brien, W. E., Norman, B. L., Kalumuck, K., Wistow, C. J., Borras, T., Nickerson, J. M. & Wawrousek, E. F. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3479–3483.
11. Nickerson, J. M., Wawrousek, E. F., Borras, T., Hawkins, J. W., Norman, B. L., Filpula, D. R., Nagle, J. W., Ally, A. H. & Piatigorsky, J. (1986) *J. Biol. Chem.* **261,** 552–557.
12. Nickerson, J. M., Wawrousek, E. F., Hawkins, J. W., Wakil, A. S., Wistow, G. J., Thomas, G., Norman, B. L. & Piatigorsky, J. (1985) *J. Biol. Chem.* **260,** 9100–9105.
13. Thomas, G., Zelenka, P., Cuthbertson, R. A., Norman, B. L. & Piatigorsky, J. (1990) *New Biol.* **2,** 903–914.
14. Head, M. W., Triplett, E. L. & Clayton, R. M. (1991) *Exp. Cell Res.* **193,** 370–374.
15. Li, X., Zelenka, P. S. & Piatigorsky, J. (1993) *Dev. Dynam.* **196,** 114–123.
16. Kondoh, H., Araki, I., Yasuda, K., Matsubasa, T. & Mori, M. (1991) *Gene* **99,** 267–271.
17. Barbosa, P., Cialkowski, M. & O'Brien, W. E. (1991) *J. Biol. Chem.* **266,** 5286–5289.
18. Parker, D. S., Wawrousek, E. F. & Piatigorsky, J. (1988) *Dev. Biol.* **126,** 375–381.
19. Hayashi, S., Goto, K., Okada, T. S. & Kondoh, H. (1987) *Genes Dev.* **1,** 818–828.
20. Kessel, M. & Gruss, P. (1990) *Science* **249,** 374–379.
21. Hill, R. E., Favor, J., Hogan, B. L. M., Ton, C. T. T., Saunders, G. F., Hanson, I. M., Prosser, J., Jordan, T., Hastie, N. D. & van Heyningen, V. (1991) *Nature (London)* **354,** 522–525, and erratum (1992) **355,** 750.
22. Ton, C. C., Hirvonen, H., Miwa, H., Wiel, M., Monaghan, P., Jordan, T., van Heyningen, V., Hastie, N., Meijers-Heijboer, H., Drechsler, M., Royer-Pokora, B. Z., Collins, F., Swaroop, A., Strong, L. C. & Saunders, G. F. (1991) *Cell* **67,** 1059–1074.
23. Walther, C. & Gruss, P. (1991) *Development (Cambridge, U.K.)* **113,** 1435–1449.
24. Glaser, T., Walton, D. S. & Maas, R. L. (1992) *Nat. Genet.* **2,** 232–239.
25. Li, H.-S., Yang, J.-M., Jacobson, R., Pasko, D. & Sundin, O. (1994) *Dev. Biol.* **162,** 181–194.
26. Zuker, C. S. (1994) *Science* **265,** 742–743.
27. Glaser, T., Jepeal, L., Edwards, J. G., Young, S. R., Favor, J. & Maas, R. (1994) *Nat. Genet.* **7,** 463–471.
28. Martha, A., Ferrell, R. E., Mintz-Hittner, H., Lyons, L. A. & Saunders, G. F. (1994) *Am. J. Hum. Genet.* **54,** 801–811.
29. Quiring, R., Walldorf, U., Kloter, U. & Gehring, W. (1994) *Science* **265,** 785–789.
30. Hanson, I. M., Fletcher, J. M., Jordan, T., Brown, A., Taylor, D., Adams, R. J., Punnett, H. H. & van Heyningen, V. (1994) *Nat. Genet.* **6,** 168–173.
31. Cvekl, A., Sax, C. M., Bresnick, E. & Piatigorsky, J. (1994) *Mol. Cell. Biol.* **14,** 7363–7376.
32. Cvekl, A., Kashanchi, F., Sax, C. M., Brady, J. N. & Piatigorsky, J. (1995) *Mol. Cell. Biol.* **15,** 653–660.
33. Richardson, J., Cvekl, A. & Wistow, G. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4676–4680.
34. Kamachi, Y. & Kondoh, H. (1993) *Mol. Cell. Biol.* **13,** 5206–5215.
35. Czerny, T., Schaffner, G. & Busslinger, M. (1993) *Genes Dev.* **7,** 2048–2061.
36. Epstein, J., Cai, J., Glaser, T., Jepeal, L. & Maas, R. (1994) *J. Biol. Chem.* **269,** 8355–8361.
37. Klement, J. F., Wawrousek, E. F. & Piatigorsky, J. (1989) *J. Biol. Chem.* **264,** 19837–19844.
38. Shapiro, D. J., Sharp, P. A., Wahli, W. W. & Keller, M. J. (1988) *DNA* **7,** 47–55.
39. Cvekl, A., Horská, K., Vlček, C. & Pačes, V. (1991) *Gene* **103,** 253–257.
40. Bresnick, E. H. & Felsenfeld, G. (1993) *J. Biol. Chem.* **268,** 18824–18834.
41. Carriere, C., Plaza, S., Martin, P., Quatannens, B., Bailly, M., Stehelin, D. & Saule, S. (1993) *Mol. Cell. Biol.* **13,** 7257–7266.
42. Sekido, R., Murai, K., Funahashi, J., Kamachi, Y., Fujisawa-Schara, A., Nabeshima, Y. & Kondoh, H. (1994) *Mol. Cell. Biol.* **14,** 5692–5700.
43. Funahashi, J., Sekido, R., Murai, K., Kamachi, Y. & Kondoh, H. (1993) *Development (Cambridge, U.K.)* **119,** 433–446.
44. Sawadogo, M. & Roeder, R. G. (1985) *Cell* **43,** 165–175.
45. Grainger, R. M. (1992) *Trends Genet.* **8,** 349–355.
46. Saha, M. S., Servetnick, M. & Grainger, R. M. (1992) *Curr. Opin. Genet. Dev.* **2,** 582–588.
47. Glaser, T., Walton, D. S., Cai, J., Epstein, J. A., Jepeal, L. & Maas, R. L. (1995) in *Molecular Genetics of Ocular Disease* (Wiley/Liss, New York), pp. 51–82.
48. Kondoh, H., Katoh, K., Takahashi, Y., Fujisawa, H., Yokoyama, M., Kimura, S., Katsuki, M., Saito, M., Nomura, T., Hiramoto, Y. & Okada, T. S. (1987) *Dev. Biol.* **120,** 177–185.
49. Li, X. & Piatigorsky, J. (1993) *Invest. Ophthamol. Visual Sci.* **34,** Suppl., 1456.
50. Piatigorsky, J. (1993) *Dev. Dynam.* **196,** 267–272.
51. Sullivan, C. H., Norman, J. T., Borras, T. & Grainger, R. M. (1989) *Mol. Cell. Biol.* **9,** 3132–3135.
52. Sullivan, C. H., O'Farrell, S. & Grainger, R. M. (1991) *Dev. Biol.* **115,** 40–50.
53. Funahashi, J., Kamachi, Y., Goto, K. & Kondoh, H. (1991) *Nucleic Acids Res.* **19,** 3543–3547.
54. Chalepakis, G., Wijnholds, J., Giese, P., Schachner, M. & Gruss, P. (1994) *DNA Cell Biol.* **13,** 891–900.
55. Gopal-Srivastava, R. & Piatigorsky, J. (1994) *Nucleic Acids Res.* **22,** 1281–1286.
56. Tomarev, S. I., Duncan, M. K., Roth, H. J., Cvekl, A. & Piatigorsky, J. (1994) *J. Mol. Evol.* **39,** 134–143.
57. Piatigorsky, J. & Wistow, G. (1991) *Science* **252,** 1078–1079.