Int. J. Dev. Biol. 40: 531-535 (1996)

Original Article

Characterization of terminally differentiated cell state by categorizing cDNA clones derived from chicken lens fibers

KAICHIRO SAWADA[1], KIYOKAZU AGATA[2] and GORO EGUCHI*

Division of Morphogenesis, Department of Developmental Biology, National Institute for Basic Biology, Okazaki, Japan

ABSTRACT To characterize a terminally differentiated state of cells at the gene expression level, a cDNA library of chicken lens fibers was analyzed. The major population of the library consisted of cDNAs encoding δ-crystallin (about 35% of the recombinants) and other crystallins (αA-, αB-, βA3/A1-, βB1-, βB2-), as well as cytoskeletal proteins (CP49, CP95), and membrane protein (MP28). These cDNA clones representing lens structural proteins known, accounted for about 60% of the library. When 96 clones were randomly selected from this library, 55 clones corresponded to the above-mentioned major class proteins. Analyses of the remaining clones indicated that many of them were expressed in a lens-specific manner at very low levels. The partial nucleotide sequence analysis of these clones revealed that two cDNAs corresponded to the genes encoding lens-type connexin, three cDNAs to the genes encoding housekeeping proteins, and some cDNAs to the genes encoding regulatory proteins. The mRNA composition in the chicken lens fiber cells indicated rather simple organization of mRNA species of this cell type, and gave scope to the possibility of full description of differentiated lens fiber cells at gene activity level.

KEY WORDS: *terminal differentiation, lens fibers, cDNA library*

Introduction

Developmental processes of biological organisms include the differentiation of various types of cells comprising specific tissues of mature individuals. It is interesting how the terminally differentiated states of cells are maintained in terms of gene activity. Description of the mRNA composition in the terminally differentiated cells should be a good standpoint to approach this subject.

Lens fibers are typical examples of terminal differentiated tissues composed of a single type of cell. A limited number of discrete proteins seem to be exclusively expressed in the lens fiber cells. The most abundant proteins are crystallins, followed by cytoskeletal and membrane-associated proteins. The cDNAs corresponding to these major classes of proteins have been isolated (Yasuda *et al.*, 1984; Okazaki *et al.*, 1985; Hejtmancik *et al.*, 1986; Peterson and Piatigorsky, 1986; Kodama *et al.*, 1990; Sawada *et al.*, 1992, 1995; Orii *et al.*, 1993; Remington, 1993). Other minor proteins were also studied but many of them might remain as unknown classes of proteins. To assess the mRNA composition of these known and unknown proteins, we made the population analysis of cDNA library using chicken lens fiber cells as a good model system to study gene activity of fully differentiated cells.

Results

Population sizes and expression of nine major genes

cDNAs of nine genes which are known to be expressed specifically in the chicken lens were used for the analysis. They were six cDNAs of genes encoding crystallins (αA-, αB-, βA3/A1-, βB1-, βB2-, and δ-crystallin), two cDNAs of genes encoding cytoskeletal proteins (CP49 and CP95) and one cDNA of a gene encoding major intrinsic protein (MP28).

Dot blot hybridizations against 96 clones randomly selected from the library were performed. Fifty-five clones showed positive signals to probes for these nine genes. Thirty-four clones contained δ-crystallin cDNA. The cDNAs of the genes encoding the other crystallins, MP28, and CP95, were detected in 1-5 clones, and CP49 cDNA was not included in the selected clones (Table 1, first row).

The population sizes of the cDNAs of these genes in the cDNA library except that encoding δ-crystallin were confirmed by plaque hybridization with a larger number of recombinants (more than 5000 plaques) (Table 1, second row). Two sets of values of the population sizes showed good agreement.

The expression of these genes in one day-old chicken lenses was also examined by mRNA dot blot hybridization (Table 1, third row). About 1 μg of poly(A)$^+$ RNA of the lens epithelium and lens

***Address for reprints**: Division of Morphogenesis, Department of Developmental Biology, National Institute for Basic Biology, Myodaijicho, Okazaki 444, Japan. FAX: 81-564.557575.e-mail: eguchi@nibb.ac.jp

Present addresses: [1]Biohistory Research Hall, Murasakicho 1-1, Takatsuki, Osaka 569-11, Japan, and [2]Laboratory of Regeneration, Department of Life Science, Himeji Institute of Technology, Akou, Hyogo 678-12, Japan.

0214-6282/96/$03.00

TABLE 1

POPULATION SIZES AND EXPRESSION OF NINE MAJOR GENES IN THE LENS FIBERS

	crystallins						cytoskeletal proteins		
	δ	αA	αB	βA3/A1	βB1	βB2	CP49	CP95	MP28
number of clones showing positive reaction among 96 clones (%)	34 (35.4)	5 (5.2)	2 (2.1)	5 (5.2)	2 (2.1)	2 (2.1)	0 (0)	4 (4.2)	1 (1.0)
population size in cDNA library %	–	4.4	2.4	5.9	2.8	3.4	0.5	3.4	0.7
mRNA dot blot hyb. lens fibers									
lens epithelium									

Population sizes in the second row were determined by plaque hybridization with ca. 5,000 recombinants. Signal intensities of mRNA dot blot hybridization were not normalized, nor reflecting the relative expression level among each gene.

fibers was blotted on nylon membranes and hybridized. All of the crystallin cDNAs reacted equally with both the lens epithelium and lens fibers except βB1-crystallin cDNA which reacted weakly with the mRNA of the lens epithelium. In contrast, all of the cytoskeletal protein cDNAs and the major intrinsic protein (MIP) cDNA reacted only with the mRNA of lens fibers.

Characterization of remaining cDNA clones

Of the 96 randomly selected clones, 41 did not react with any probes for the nine genes above. Thirty-eight of them could be recloned in plasmid vector and were examined as examples of unknown clones in the lens fiber cDNA library. The mutual cross-reactivities, levels of expression in four tissue types (lens epithelium, lens fibers, liver, and neural retina) and the population sizes in the cDNA library were examined. Results are summarized in Table 2. Except for the cross-reactivity between clones No. 47 and No. 84, they represented unique sequences. Thirteen clones were detected in both lens and non-lens tissues, and showed variable abundance in the lens. Other 25 were detected in lens tissues but not in either liver or neural retina. Of these 25, five were expressed in both the lens epithelium and lens fibers and the other 20 were expressed specifically in lens fibers. None of these lens-specific clones accounted for more than 1% of the cDNA library. The population size of three clones (No. 10, No. 47 and No. 84) was 0.2% of the cDNA library. Other 22 clones represented less abundant mRNA species (0.1% or less of the cDNA library).

The partial DNA sequences of some of these unknown clones showed high similarities to reported sequences in the available databases such as Genbank. Results are summarized in Table 3. Some of these clones were revealed to contain cDNAs of genes encoding presumptive regulatory proteins. They were nuclear proteins (BBC1: Adams, S.M. *et al.*, 1992; dbpB/YB-1: Didier *et al.*, 1988; Sakura *et al.*, 1988), a protein containing ankyrin repeats (V1 protein: Taoka *et al.*, 1992), a translationally controlled protein (p23: Chitpatima *et al.*, 1988; Gross *et al.*, 1989), and a TGFβ-induced protein containing leucine-zipper motif (TSC-22, Shibanuma *et al.*, 1992). Relatively low but significant similarities were observed between a sequence of clone No. 65 and that of a gene encoding RNase H (Mushegian *et al.*, 1994), and between a sequence of clone No. 95 and that of a gene encoding a protein containing armadillo repeats (p120 protein: Reynolds *et al.*, 1992). cDNA clones representing these presumptive regulatory proteins were found at very low levels (0.1% or below) except for clones representing dbpB/YB-1 (1.4%) and TSC-22 (0.2%). cDNAs of genes encoding housekeeping proteins were found in three clones (No. 9: mitochondrial ATPase, No. 53: α-tubulin, and No. 72: elongation factor-1α). Two clones (No. 47 and No. 84) contained cDNAs of genes encoding lens-type connexin (Connexin 50: White *et al.*, 1992; Connexin 45.6: Genbank L24799), which was another constructive protein of lens fibers. cDNA of two clones (No. 15 and No. 68) contained a sequence encoding part of the 28S rRNA, which was thought to be a contaminant in the cDNA library.

Discussion

We analyzed a cDNA library of chicken lens fibers as an example of terminally differentiated cells. In Table 4, clones of nine known genes and 38 other clones examined were categorized according to the population size in the cDNA library and the expression specificity in the lens and other tissues. Distinctive features of the composition of the chicken lens fiber cDNA library indicated in this study were as follows. (i) Most of the cDNAs in the library encoded lens-specific proteins, such as crystallins, CP95, CP49, MP28, and lens-type connexin (0.2%-35% of the library). The sum of these cDNAs is thought to account for about 60% of the library. (ii) In contrast, the populations of cDNAs of housekeeping genes (genes encoding α-tubulin, mitochondrial ATPase, and EF-1α) were relatively small (0.06%-0.3%). (iii) Genes of many of the unidentified cDNA clones were expressed in a lens-specific manner. Expression of most of them seemed to be up-regulated according to the differentiation of lens fiber cells from lens epithelial cells. (iv) Several clones encoding presump-

tive regulatory proteins were found in a class of cDNAs expressed at very low levels (0.1% or below).

In the case of the human brain as a tissue mainly composed of terminally differentiated cells, the first and second abundantly expressed genes are the β-actin gene (0.6% of the nuclear-encoded mRNAs) and the myelin basic protein gene (0.5%) (Adams M.D. *et al.*, 1992). Comparing to the brain, the extraordinary abundance of mRNAs of major lens proteins in the lens fiber cell is obvious. mRNAs representing many species of regulatory factors of lens fiber differentiation are also expressed though the expression level of each mRNA is very low.

The major lens-specific proteins have been studied well as markers for terminal differentiation. The distinct spatiotemporal patterns of their expression in the lens were known (e.g. Papaconstantinou, 1967). Furthermore, nonlenticular expression of some classes of crystallins was observed in several tissues (e.g. Agata *et al.*, 1983; Iwaki *et al.*, 1989; Head *et al.*, 1991; Sawada *et al.*, 1993). These findings showed that transcription of each gene encoding a major lens protein could be regulated independently, and that many different regulatory factors are probably involved in transcriptional regulation of each gene.

Some of the factors involved in the regulation of crystallin gene expression have already been identified by analysis of binding specificities to enhancer elements of each gene (Nakamura *et al.*, 1990; Matsuo *et al.*, 1991; Funahashi *et al.*, 1993), and binding sites for many regulatory factors including Pax-6, USF, CREB, and AP-1 proteins were also identified in the region upstream of the αA-crystallin gene (Cvekl *et al.*, 1994). These findings also suggest that many different regulatory mechanisms and factors are involved in lens cell differentiation.

To clarify the terminally differentiated cell state including these multifarious mechanisms, the survey of all genes expressed in lens fiber cells is thought to be effective. The 'Human Gene Anatomy' project reported 83 million nucleotide sequences of human cDNA libraries. However, of 88,000 distinct sequences, only about 10,000 could be identified as known genes (Adams *et al.*, 1995). In contrast, the present study demonstrates that 60% of the clones of the chicken lens fiber cDNA library could be identified as a cDNA of one of the limited numbers of genes, and that cDNAs of many regulatory factors might account for some of the remaining 40% of the library. Because the averaged population size of the minor cDNAs in this study was calculated as about 0.04%, only 1,000 different cDNAs are estimated to comprise this 40% of the cDNA library. Since more minor cDNAs, which could not be detected in this study, may exist, the actual number of different mRNAs expressed may be up to double. Even in such a case, it is easy to capture the cDNAs of novel minor genes which are expressed specifically in lens fiber cells by effective subtraction of the major cDNAs. Although it is not certain how many of these clones might encode factors involved in the regulation of lens fiber differentiation, we will be able to

TABLE 2

POPULATION SIZES AND EXPRESSION LEVELS OF 38 UNHYBRIDIZED CLONES

clone No.	mRNA Liv	dot NR	blot LF	hyb. LE	population in cDNA library(%)	length (kb)	accession No.
4	-	-	++	+	0.05	1.4	D26311
6	-	+	++	+	0.03	0.8	D26312
8	-	-	++	-	0.04	1.4	D26313
9	++	+	+	-	0.06	0.2	D26314
10	-	-	+	-	0.2	1.6	D26316
12	-	-	+	-	0.03	0.7	D26317
14	++	+	++	+	0.1	0.7	D26318
15	+	+	+	+	1.0	1.5	D26319
16	-	-	+	-	0.07	0.4	D26320
18	-	-	+	-	0.01	1.0	D26321
19	-	-	+	-	0.06	0.6	D26322
21	-	-	+	-	0.01	1.3	D26323
22	-	+	++	+	0.01	1.4	D26324
23	-	-	++	-	0.01	1.8	D26325
30	-	-	+	-	0.1	3.7	D26326
31	-	-	+	-	0.01	0.8	D26327
34	+	+	++	+	1.4	1.5	D26328
38	-	-	+	-	0.02	1.4	D26329
39	-	-	+	-	0.04	1.0	D26330
42	-	-	+	-	0.06	2.8	D26331
45	-	-	+	-	0.03	0.7	D26332
47	-	-	+	-	0.2	0.8	D26333
48	-	-	+	-	0.02	1.3	D26334
52	-	+	+	+	0.2	1.5	D49740
53	-	+	+	+	0.3	1.4	D26336
56	-	+	+	+	0.03	1.1	D26337
58	-	-	++	+	0.09	2.7	D26315
61	-	-	+	+	0.04	1.3	D26338
63	+	-	+	++	0.04	0.9	D26339
65	-	-	+	+	0.01	1.3	D36340
68	+	+	+	++	1.4	1.0	D26341
72	+	+	+	++	0.2	0.7	D26342
74	-	-	+	-	0.01	0.9	D26343
77	-	-	+	-	0.01	1.2	D26344
81	-	-	+	-	0.01	0.1	D26345
84	-	-	++	+	0.2	1.8	D26346
90	-	-	+	-	0.01	0.4	D26347
95	-	+	++	+	0.01	2.0	D49741

Abbreviations: Liv, Liver; NR, neural retina; LE, lens epithelium; LF, lens fibers. The nucleotide sequences will appear in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases with the above accession numbers. Population sizes were determined by plaque hybridization with ca. 5,000 recombinants (for clones which accounted for more than 0.1%) or more than 10,000 recombinants (for clones which accounted for 0.1% or below).

TABLE 3

RESULTS OF THE SIMILARITY SEARCH OF UNHYBRIDIZED CLONES

clone No.	expression in tissues	population in cDNA library	protein encoded by homolog & similarity		
14	non-specific	0.1%	BBC1	195aa/211aa	(92%)
34	non-specific	1.4%	dbpB/YB-1	302aa/326aa	(93%)
30	lens fiber	0.1%	V1 protein	113aa/118aa	(96%)
6	non-specific	0.03%	p23	155aa/172aa	(90%)
52	non-specific	0.2%	TSC-22	95aa/143aa	(66%)
65	lens	0.01%	RNase H	84aa/293aa	(29%)
95	non-specific	0.01%	p120	36aa/112aa	(32%)
9	non-specific	0.06%	mt ATPase	161bp/163bp	(99%)
53	non-specific	0.3%	α-tubulin	138bp/142bp	(96%)
72	non-specific	0.2%	EF-1α	162bp/190bp	(85%)
47	lens fiber	0.2%	Connexin 45.6	234aa/235aa	(99%)
84	lens	0.2%	Connexin 50	162bp/296bp	(55%)
15	non-specific	1.0%	28S rRNA	109bp/114bp	(96%)
68	non-specific	1.4%	28S rRNA	100bp/108bp	(93%)

Sequences of nucleotides (bp) or deduced amino acids (aa) are used to calculate the similarities.

narrow down the number of candidates with further analysis. The approach described here is considered to be very useful for the investigation of various biological phenomena of terminal differentiation, and the lens is one of the best materials to analyze for that purpose.

TABLE 4

SUMMARY OF THE ANALYSIS OF THE CHICKEN LENS FIBER cDNA LIBRARY

rank of population size in cDNA library	expression patterns		
	LE+LF	LF	non-specific
>10%	δ-crystallin		
>1% & ≤10%	αA-, αB-, βA3/A1-, βB1-, βB2-crystallin	CP95	rRNA(15,68), dbpB/YB-1(34)
>0.1% & ≤1%	Cx(84)	MP28, CP49 CX(47) 10	TSC-22(52), α-tubulin(53), EF-1α(72)
≤0.1%	RNaseH(65) 4, 58, 61	V1p(30) 8, 12, 16, 18, 19 21, 23, 31, 38, 39 42, 45, 48, 74, 77 81, 90	p23(6), mtATPase(9), BBCl(14), p120(95), 22, 56, 63

Abbreviations: LE, lens epithelium; LF, lens fibers. cDNAs of nine genes known were categorized according to results shown in Table 1, and represented by names of encoded proteins. cDNAs of other genes were categorized according to the results shown in Table 2. Clones listed in Table 4 are represented by the names of homologs with clone numbers. Others are represented by their clone numbers.

Materials and Methods

RNA dot analysis

Lens fibers, lens epithelia, neural retinae and livers were isolated from one day-old chickens. Tissues were homogenized in a solution of 0.1 M Tris-HCl pH 9.0 and 1% SDS. Nucleic acids were extracted by phenol and phenol-chloroform saturated with 0.1 M Tris-HCl pH 9.0, and RNA was purified by centrifugation on 5.7 M CsCl. Poly(A)$^+$ RNA was purified using oligo-dT-conjugated latex, Oligotex (JSR-Roche). Purified poly(A)$^+$ RNA was blotted on the nylon membrane using a dot blot apparatus (Bio-Rad) (1 μg per dot).

Conventional procedures of dot blot hybridization (White and Bancroft, 1982) were performed.

Analysis of cDNAs

The cDNA library of chicken lens fibers was constructed as described previously (Sawada *et al.*, 1992). Plaque hybridization was performed according to the method of Benton and Davis (1977).

For sequencing, *Eco*RI fragments derived from the selected recombinants in the cDNA library were subcloned into the pTZ19R vector. The dideoxy-termination method (Sanger *et al.*, 1977) was used with a Sequenase DNA sequencing kit (USB).

Acknowledgments

We wish to thank Dr. A.R. Mushegian (Univ. of Washington), Dr. H. Aoyama and Dr. T.C. Takahashi (Biohistory Research Hall, Japan) for helpful suggestions, and Ms. C. Takagi and Ms. H. Urai for technical support. This work was supported by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan to Dr. G. Eguchi, and by Special Coordination Funds for Promoting Science and Technology from the Science and Technology Agency of Japan to Dr. K. Agata.

References

ADAMS, M.D., DUBNICK, M., KERLAVAGE, A.R., MORENO, R., KELLEY, J.M., UTTERBACK, T.R., NAGLE, J.W., FIELDS, C. and VENTER, J.C. (1992). Sequence identification of 2,375 human brain genes. *Nature 355:* 632-634.

ADAMS, M.D., KERLAVAGE, A.R., FLEISCHMANN, R.D., FULDNER, R.A., BULT, C.J., et al. (1995). Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence. *Nature 377 (Suppl.):* 3-174.

ADAMS, S.M., HELPS, N.R., SHARP, M.G.F., BRAMMAR, W.J., WALKER, R.A. and VARLEY, J.M. (1992). Isolation and characterization of a novel gene with differential expression in benign and malignant human breast tumours. *Human Mol. Genet. 1:* 91-96.

AGATA, K., YASUDA, K. and OKADA, T.S. (1983). Gene coding for a lens-specific protein, δ-crystallin, is transcribed in non lens tissues of chicken embryo. *Dev. Biol. 100:* 222-226.

BENTON, W.D. and DAVIS, R.W. (1977). Screening λgt recombinant clones by hybridization to single plaques *in situ*. *Science 196:* 180.

CHITPATIMA, S.T., MAKRIDES, S., BANDYOPADHYAY, R. and BRAWERMAN, G. (1988). Nucleotide sequence of a major messenger RNA for a 21 kilodalton polypeptide that is under translational control in mouse tumor cells. *Nucleic Acids Res. 16:* 2350.

CVEKL, A., SAX, C.M., BRESNICK, E.H. and PIATIGORSKY, J. (1994). A complex array of positive and negative elements regulates the chicken αA-crystallin gene: involvement of Pax-6, USF, CREB and/or CREM, and AP-1 proteins. *Mol. Cell. Biol. 14:* 7363-7376.

DIDIER, D.K., SCHIFFENBAUER, J., WOULFE, S.L., ZACHEIS, M. and SCHWARTZ, B.D. (1988). Characterization of the cDNA encoding a protein binding to the major histocompatibility complex class II Y box. *Proc. Natl. Acad. Sci. USA 85:* 7322-7326.

FUNAHASHI, J., SEKIDO, R., MURAI, K., KAMACHI, Y. and KONDOH, H. (1993). δ-Crystallin enhancer binding protein δEF1 is a zinc finger-homeodomain protein implicated in postgastrulation embryogenesis. *Development 119:* 433-446.

GROSS, B., GAESTEL, M., BOHM, H. and BIELKA, H. (1989). cDNA sequence coding for a transcriptionally controlled human tumor protein. *Nucleic Acids Res. 17:* 8367.

HEAD, M.W., PETER, A. and CLAYTON, R.M. (1991). Evidence for the extralenticular expression of members of the β-crystallin gene family in the chick and a comparison with δ-crystallin during differentiation and transdifferentiation. *Differentiation 48:* 147-156.

HEJTMANCIK, J.F., THOMPSON, M.A., WISTOW, G. and PIATIGORSKY, J. (1986). cDNA and deduced protein sequence for the βB1-crystallin polypeptide of the chicken lens. *J. Biol. Chem. 261:* 982-987.

IWAKI, T., KUME-IWAKI, A., LIEM, R.K.H. and GOLDMAN, J.E. (1989). αB-crystallin is expressed in non-lenticular tissues and accumulates in Alexander's disease brain. *Cell 57:* 71-78.

KODAMA, R., AGATA, K., MOCHII, M. and EGUCHI, G. (1990). Partial amino acid sequence of the major intrinsic protein (MIP) of the chicken lens deduced from the nucleotide sequence of a cDNA clone. *Exp. Eye Res. 50:* 737-741.

MATSUO, I., KITAMURA, M., OKAZAKI, K. and YASUDA, K. (1991). Binding of a factor to an enhancer element responsible for the tissue-specific expression of the chicken αA-crystallin gene. *Development 113:* 539-550.

MUSHEGIAN, A.R., EDSKES, H.K. and KOONIN, E.V. (1994). Eukaryotic RNase H shares a conserved domain with caulimovirus proteins that facilitate translation of polycistronic RNA. *Nucleic Acids Res. 22:* 4163-4166.

NAKAMURA, T., DONOVAN, D.M., HAMADA, K., SAX, C.M., NORMAN, B., FLANAGAN, J.R., OZATO, K., WESTPHAL, H. and PIATIGORSKY, J. (1990). Regulation of the mouse αA-crystallin gene: isolation of a cDNA encoding a protein that binds to a cis sequence motif shared with the major histocompatibility complex class I gene and other genes. *Mol. Cell Biol. 10:* 3700-3708.

OKAZAKI, K., YASUDA, K., KONDOH, H. and OKADA, T.S. (1985). DNA sequences responsible for tissue-specific expression of a chicken α-crystallin gene in mouse lens cells. *EMBO J. 4:* 2589-2595.

ORII , H., AGATA, K., SAWADA, K., EGUCHI, G. and MAISEL, H. (1993). Evidence that the chick lens cytoskeletal protein CP49 belongs to the family of intermediate filament proteins. *Curr. Eye Res. 12:* 583-588.

PAPACONSTANTINOU, J. (1967). Molecular aspects of lens cell differentiation. *Science 156:* 338-346.

PETERSON, C.A. and PIATIGORSKY, J. (1986). Preferential conservation of the globular domains of the βA3/A1-crystallin polypeptide of the chicken eye lens. *Gene 45:* 139-147.

REMINGTON, S.G. (1993). Chicken filensin: a lens fiber cell protein that exhibits sequence similarity to intermediate filament proteins. *J. Cell Sci. 105:* 1057-1068.

REYNOLDS, A.B., HERBERT, L., CLEAVELAND, J.L., BERG, S.T. and GAUT, J.R. (1992). p120, a novel substrate of protein tyrosine kinase receptors and of p60v-src, is related to cadherin-binding factors β-catenin, plakoglobin and armadillo. *Oncogene 7:* 2439-2445.

SAKURA, H., MAEKAWA, T., IMAMOTO, F., YASUDA, K. and ISHII, S. (1988). Two human genes isolated by a novel method encode DNA-binding proteins containing a common region of homology. *Gene 73:* 499-507.

SANGER, F., NICKLEN, S. and COULSON, A.R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA 12:* 5463-5467.

SAWADA, K., AGATA, K. and EGUCHI, G. (1992). Crystallin gene expression in the process of lentoidogenesis in cultures of chicken lens epithelial cells. *Exp. Eye Res. 55:* 879-887.

SAWADA, K., AGATA, K., EGUCHI, G., QUINLAN, R. and MAISEL, H. (1995). The predicted structure of chick lens CP49 and a variant thereof, CP49ins, the first vertebrate cytoplasmic intermediate filament protein with a lamin-like insertion in helix 1B. *Curr. Eye Res. 14:* 545-553.

SAWADA, K., AGATA, K., YOSHIKI, A. and EGUCHI, G. (1993). A set of anti-crystallin monoclonal antibodies for detecting lens specificities: β-crystallin as a specific marker for detecting lentoidogenesis in cultures of chicken lens epithelial cells. *Jpn. J. Opthalmol. 37:* 355-368.

SHIBANUMA, M., KUROKI, T. and NOSE, K. (1992). Isolation of a gene encoding a putative leucine zipper structure that is induced by transforming growth factor β1 and other growth factors. *J. Biol. Chem. 267:* 10219-10224.

TAOKA, M, YAMAKUNI, T., SONG, S-Y., YAMAKAWA, Y., SETA, K., OKUYAMA, T. and ISOBE, T. (1992). A rat cerebellar protein containing the cdc10/SWI6 motif. *Eur. J. Biochem. 207:* 615-620.

WHITE, B.A. and BANCROFT, F.C. (1982). Cytoplasmic dot hybridization. Simple analysis of relative mRNA level in multiple small cell or tissue samples. *J. Biol. Chem. 257:* 8569.

WHITE, T.W., BRUZZONE, R., GOODENOUGH, D.A. and PAUL, D.L. (1992). Mouse Cx50, a functional member of the connexin family of gap junction proteins, is the lens fiber protein MP70. *Mol. Biol. Cell 3:* 711-720.

YASUDA, K., NAKAJIMA, N., ISOBE, T., OKADA, T.S., and SHIMURA, Y. (1984). The nucleotide sequence of a complete chicken δ-crystallin cDNA. *EMBO J. 3:* 1397-1402.

Received: December 1995
Accepted for publication: February 1996