Convergent Evolution of Crystallin Gene Regulation in Squid and Chicken: The AP-1/ARE Connection

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Abstract. Previous experiments have shown that the minimal promoters required for function of the squid SL20-1 and SL11 crystallin genes in transfected rabbit lens epithelial cells contain an overlapping AP-1/antioxidant responsive element (ARE) upstream of the TATA box. This region resembles the PL-1 and PL-2 elements of the chicken βB1-crystallin promoter which are essential for promoter function in transfected primary chicken lens epithelial cells. Here we demonstrate by site-directed mutagenesis that the AP-1/ARE sequence is essential for activity of the squid SL20-1 and SL11 promoters in transfected embryonic chicken lens cells and fibroblasts. Promoter activity was higher in transfected lens cells than in fibroblasts. Electrophoretic mobility shift and DNase protection experiments demonstrated the formation of numerous complexes between nuclear proteins of the embryonic chicken lens and the AP-1/ARE sequences of the squid SL20-1 and SL11 crystallin promoters. One of these complexes comigrated and cross-competed with that formed with the PL-1 element of the chicken βB1-crystallin promoter. This complex formed with nuclear extracts from the lens, heart, brain, and skeletal muscle of embryonic chickens and was eliminated by competition with a consensus AP-1 sequence. The nonfunctional mutant AP-1/ARE sequences did not compete for complex formation. These data raise the intriguing possibility that entirely different, nonhomologous crystallin genes of the chicken and squid have convergently evolved a similar cis-acting regulatory element (AP-1/ARE) for high expression in the lens.

Key words: Lens — Crystallin — Squid — Chicken — Gene — Regulation — AP-1 — Evolution

Introduction

Complex eyes containing photoreceptors, a pigmented epithelium, and a transparent lens were formed independently in the course of vertebrate and invertebrate evolution (Land 1984; Land and Fernald 1992). Among the invertebrates, cephalopod (squid, octopus, and cuttlefish) eyes are best known for their striking similarity to those of vertebrates (Packard 1972). In both cases, a transparent lens is used to focus incoming light onto photoreceptor cells. The major soluble proteins in the eye lens of vertebrates and invertebrates are called crystallins. Vertebrate crystallins are a very diverse group of proteins which often differ in composition between different species (Wistow and Piatigorsky 1988; de Jong et al. 1989). Some crystallins are present in the lenses of all vertebrates (the α and β/γ-crystallins), while others, generally related or identical to metabolic enzymes, are restricted to certain species (the taxon-specific crystallins). S-crystallins are the major lens proteins of squids (Siezen and Shaw 1982). These are not present in vertebrate lenses and are related to the metabolic enzyme, glutathione S-transferase (GST) (Wistow and Piatigorsky 1987; Tomarev and Zinovieva 1988; Tomarev et al. 1992, 1993).

The basis for recruiting a lens crystallin during evo-
olution is not known. The fact that many, if not all, of the crystallins are either derived from or identical to proteins providing stress protection or detoxification functions suggests that their recruitment may have involved, at least during the early stages of selection, the induction of gene expression by some form of metabolic stress or environmental signal (Piatigorsky and Wistow 1989; de Jong et al. 1989; Wistow and Kim 1991). The possibility has also been advanced that transcription factors required for the optimal expression of crystallin genes in the lens may have common properties despite differences in the nature of the encoded crystallins (Piatigorsky 1992). If this idea is correct, one would expect to find similar transcription factors utilized for high expression of different crystallin genes in the lens of the same and different species.

In the course of our investigations on the squid, we noted that a consensus AP-1 binding sequence overlapping an antioxidant response element (ARE) was present in the minimal functional 5' flanking sequences of the SL20-1 and SL11 crystallin genes. This element is required for activity of the squid promoters fused to the bacterial chloramphenicol acetyltransferase (CAT) gene in transfected N/N1003A rabbit lens epithelial cells (Tomarev et al. 1992). This was of special interest since AP-1 has been implicated in the expression of numerous crystallin genes in vertebrates (see Piatigorsky and Zelenka 1992) and both AP-1 and ARE are involved in the induction of GST genes by oxidative stress in mammals (Daniel 1993). Moreover, the AP-1/ARE element in the squid promoters resembles the PL-1 and PL-2 functional elements of the chicken βB1-crystallin promoter (Roth et al. 1991). Consequently, we used site-directed mutagenesis, transfection experiments, electrophoretic mobility shift assays (EMSA), and DNase I footprint analysis to compare the functional requirements and binding properties of the AP-1/ARE sequences found in the squid promoters with the PL-1 element of the chicken βB1-crystallin promoter in chicken lens cells. The results indicate that the squid AP-1/ARE sequences are essential for promoter activity in transfected chicken lens cells and fibroblasts. Moreover, the squid AP-1/ARE sequences form a complex similar to that formed with the PL-1 element of the chicken βB1-crystallin promoter with a chicken lens nuclear extract. These data are consistent with the possibility that the SL20-1 and SL11 crystallin genes of the squid have at least one functional element in common with that of the βB1-crystallin gene of the chicken.

Materials and Methods

Nucleic Acid Isolation. Plasmid DNAs were isolated using Qiagen columns (QIAGEN Inc.). For transfection, plasmid DNAs were purified by the alkaline method (Birnboim and Doly 1979) and subsequently banded twice in a cesium chloride/ethidium bromide density gradient. RNA was isolated by the acidic guanidium thiocya-

nate–phenol–chloroform extraction method (RNazol B, Cinna/Bio-tec; Chomczynski and Sacchi 1987) from transfected and nontransfected primary lens epithelial cells.

Recombinant DNA Constructions. Plasmid DNAs containing squid S-crystallin gene promoter fragments coupled to the bacterial chloramphenicol acetyltransferase (CAT) reporter gene were described previously (Tomarev et al. 1992). Site-directed mutations were introduced into the squid S-crystallin promoters by using the oligonucleotide-directed mutagenesis method as described by the manufacturer (Amersham Corp.). All constructs were confirmed by dideoxynucleotide sequencing (Sanger et al. 1977) using Sequenase version 2.0 (U.S. Biochemical).

Transfection. Transfections were performed on patches of primary lens epithelial (PLE) cells obtained from 14-day-old chicken embryos (Borras et al. 1988). A minimum of two separate experiments were performed in duplicate for each construct tested. The test plasmid (10 µg) was cotransfected with a control SV40 promoter/β-galactosidase plasmid (pCH110) (Herbomel et al. 1984) (2 µg) as a calcium phosphate precipitate as described elsewhere (Dubin et al. 1989). Cells were assayed for CAT activity by the fluor-diffusion method (Neumann et al. 1987) and for β-galactosidase activity (Borras et al. 1988) 48 h after transfection.

Primer Extension Analysis. Primer extension analysis was performed with 50–75 µg of total RNA from transfected PLE cells. Primer 3761 (5'-CAACGGTGGTATACGACTT-3'), whose 3'-end corresponds to CAT gene position +15, was used. The primer was radioactively labeled with [γ-32P]ATP (7,000 Ci/mmol, INC) using T4 polynucleotide kinase (BRL). The labeled oligonucleotide primer was mixed with RNA, heated at 68 °C for 5 min, and incubated at 37 °C for 30 min to 1 h after addition of 10–20 units of AMV reverse transcriptase (Stratagene). RNA was hydrolyzed by NaOH treatment, and extended cDNAs were extracted with phenol-chloroform and ethanol precipitated. cDNAs were analyzed on 6% polyacrylamide/7 M urea gels. Sequencing reaction ladders generated from primer 3761 were used as a size marker.

Nuclear Extracts, Gel Mobility Shift Assays, and DNase I Footprinting. All synthetic oligonucleotides were synthesized on an Applied Biosystem 380A DNA synthesizer and purified on an 8% denaturing polyacrylamide gel. The sequences of the sense strand oligonucleotides used as probes and competitors were as follows:

SL20-1: 5'-ATTGTTTTAAGGACTCATCTATT-3'
SL11: 5'-AGGCCACCGTGACTCATCAAAAGAGATAACA-3'
PL-1: 5'-GGATGTGATGACTGGGCCGCCGCA-3'
SL20-1M6A: 5'-ATTGTATACGACACTCATCTATT-3'
SL11M6A: 5'-AGGCCACCGTGACTCATCAAAAGAGATAACA-3'
PL-1M6A: 5'-GGATGTGATGACTGGGCCGCCGCA-3'
ARE: 5'-TGCATAATTGTGACAAAGGACACTTTCGGG-3'
Rat AP-1: 5'-CGAGGTTGTCTGTCACTGCTCTTCA-3'
Chicken AP-1: 5'-CTGCTTCTCAGGACTGACGACGACA-3'

Oligodeoxynucleotides were labeled by using [γ-32P]ATP (ICN) and polynucleotide kinase to a specific activity of about 5 × 107 cpm/µg DNA; unincorporated nucleotides were removed by passage through G-25 spin columns. Double-stranded labeled probes were prepared by annealing with a threefold molar excess of the unlabeled complementary strand. Nonradioabeled competitors were prepared by annealing equimolar amounts of the complementary oligonucleotides. Nuclear extracts were prepared from the indicated tissues of 14-day-old embryonic chickens (Shapiro et al. 1988). Extracts were titrated with various oligonucleotides within the chicken eآA-crystallin enhancer (~162 to ~88) and probes containing Sp1 (GGGGCGCGCT) and octomere (ATGCAAAAT) binding sites to control for the quality of