Expression of Chx10 and Chx10-1 in the developing chicken retina

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Abstract

We have isolated full-length cDNAs of chick Chx10 and Chx10-1, two members of the paired type homeobox/CVC gene family. A comparison of sequences suggests that Chx10 is closely related to Alx/Vsx-2 and Vsx-2 of zebrafish and goldfish, respectively; while Chx10-1 is closely related to Vsx-1 of zebrafish and goldfish. Chx10 and Chx10-1 are expressed in the early retinal neuroepithelium, but not in the pigment epithelium and lens. The expression of Chx10 is present in most retinal neuroblasts, while Chx10-1 exhibits a novel pattern along the nasotemporal border. In the differentiating retina, both Chx10 and Chx10-1 are restricted to bipolar cells and are maintained at a low level in bipolar cells of the mature retina. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Results

Chx10 is a paired type homeobox gene responsible for the mouse mutation, ocular retardation (or) (Burmeister et al., 1996). The developmental defects of or mice indicate that Chx10 is essential for retinal cell proliferation and for bipolar cell development. In addition to the paired type homeodomain, Chx10 contains a highly conserved region, the CVC domain, located immediately carboxy terminal to the homeodomain (Svendsen and McGhee, 1995). A Chx10 ortholog has been found in C. elegans, fish, chicken and mouse. Two Chx10 orthologs have been isolated from goldfish (Vsx-1 and Vsx-2) (Levine et al., 1994, 1997) and zebrafish (Vsx-1 and Alx/Vsx2) (Barabino et al., 1997; Passini et al., 1998a,b). However, only one ortholog has been reported in higher vertebrates (Liu et al., 1994; Belecky-Adams et al., 1997). Here we report the isolation and sequence of full length cDNAs corresponding to two chick Chx10 orthologs, Chx10 and Chx10-1. Their detailed retinal expression patterns are also presented.

The cDNAs were isolated from a chick embryonic cDNA library. The predicted full length chick Chx10 protein contains 377 amino acid residues and is similar to the partial chick Chx10 sequence published by Belecky-Adams et al. (1997). The chick Chx10 gene also makes an alternatively spliced product which encodes a protein containing an extra 21 amino acids inserted into the CVC domain (data not shown). This alternative splicing feature is evolutionarily conserved, in that zebrafish Alx/Vsx-2 also produces an alternatively spliced product containing a 21 amino acid insertion in the CVC domain (Barabino et al., 1997). Thirteen out of these 21 amino acid residues are identical between chick Chx10 and Alx/Vsx-2 (data not shown).

The predicted Chx10-1 protein contains 350 amino acids. A sequence comparison shows that both its homeodomain and CVC domain are highly similar to the corresponding domains of Vsx-1 and Vsx-2 of goldfish, Vsx-1 and Alx/Vsx-2 of zebrafish, Chx10 of chick and mouse, and Ceh-10 of C. elegans (Fig. 1B,C). Outside of these two domains, Chx10-1 is also similar to Vsx-1, suggesting that Chx10-1 may be a homolog of fish Vsx-1 genes (Fig. 1A).

The expression patterns of chick Chx10 and Chx10-1 were examined by in situ hybridization from stage 8 embryos to 1-month-old post-hatch chicks. Neither Chx10 nor Chx10-1 mRNAs were detected on embryos younger than stage 10. By stage12, the expression of Chx10 and Chx10-1 was first observed throughout the invaginating optic vesicles (Fig. 2A,G, respectively). At this early time point, the expression of both genes was restricted to the presumptive neural retina (data not shown and Fig. 2M). Throughout all stages examined, their expression remained restricted to the neuroretina (data not shown, Fig. 2N,O). Chx10 expressed uniformly throughout the neural retina from stage 14 to stage 18.
Chx10-1 expression was restricted to a subset of retinoblasts beginning at approximately stage 14, when the optic cup forms (Fig. 2H,N). By stage 15, the retinal expression of Chx10-1 was restricted to the border of the nasal and temporal domains (Fig. 2I,O) and was highly concentrated at the dorsal and ventral margins of this nasotemporal stripe of expression (Fig. 2I). This unique spatial pattern of Chx10-1 in retina persisted until embryonic day 3.5 (E3.5) (Fig. 3F).

In addition to the expression in the retina, expression of both genes was observed in the spinal cord. By stage 12, Chx10-1 was first detected in a subset cells in the ventral spinal cord (Fig. 2G arrow), presumably a subset of interneuron precursors. The expression domain extended rostrally to the hindbrain at stage 14 (Fig. 2H,N arrows). The signal of Chx10-1 along the ventral spinal cord and hindbrain intensified at stage 15 and persisted beyond stage 20 (Fig. 2J,L and data not shown). The Chx10 gene also was expressed in the spinal cord in a pattern similar to that of Chx10-1. However, the onset of expression of Chx10 did not occur until stage 15 (Fig. 2D arrow). Its expression level was much lower than that of Chx10-1 (compare Fig. 2D,J,F,L).

The retinal expression patterns of Chx10 and Chx10-1 were followed in older embryos by in situ hybridization on sections (Fig. 3). At E3.5 and E4, Chx10 was expressed
uniformly throughout the retinal neuroepithelium (Fig. 3A–C), as also was seen in younger embryos (Fig. 2). At E6, Chx10-negative cells were detectable in the vitreal region, most likely ganglion cells, and in the scleral region, most likely in photoreceptors (Fig. 3E arrows). From E9 to E14, a high level of expression of Chx10 was seen only in the outer half of the inner nuclear layer (INL), most likely in bipolar cells (Fig. 3K,L,M). At E19, just before hatching, the
Fig. 3. In situ hybridization of sections of chick Chx10 (A–E,K–O) and Chx10-1 (F–J,P–T) on E3.5 (A,F) and E4 (B,C,G,H) chick eyes, E6 (D,E,I,J), E9 (K,P), E11 (L,Q), E14 (M,R), E19 (N,S), and P30 (O,T) chick retinae. High magnification images indicated in boxed areas in (B,G,D,I) are shown in (C,H,E,J), respectively. The retinae are oriented in nasal top and temple down direction in (A,B,D,F,G,I). le, lens; ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner plexiform layer; GC, ganglion cells.
expression level of Chx10 in the INL decreased (Fig. 3N). At 1-month post-hatch (P30), a low level of Chx10 was maintained in bipolar cells (Fig. 3O). No expression was observed in photoreceptor cells, the inner half of the INL, or in ganglion cells from E9 to P30.

The nasotemporal stripe of Chx10-1 seen in younger embryos persisted in the E3.5 retina (Fig. 3F). By E4, the Chx10-1 expression domain expanded into both the nasal and temporal regions (Fig. 3G). Unlike the uniform expression of Chx10, some cells expressed a much higher level of Chx10-1 than the others (Fig. 3H). By E6, expression of Chx10-1 extended throughout the retina, resulting in a similar pattern to that of Chx10 (Fig. 3I,J) and the pattern remained similar until E19 (Fig. 3P–S). Post-hatch, Chx10-1 decreased to a much lower level than Chx10 in bipolar cells at P30 (Fig. 3T).

2. Methods

2.1. Isolation of chick Chx10 and Chx10-1 cDNAs

The Chx10 partial cDNA sequence was isolated as a cross hybridizing clone during a screen of a chick E6-E8 retinal library using the homeodomain region of the chicken Rax gene as a probe. This partial Chx10 clone was used to isolate full-length Chx10 and Chx10-1 cDNAs. The sequence of Chx10 and Chx10-1 have been submitted to GenBank under the accession numbers AF178671 and AF178670, respectively.

2.2. In situ hybridization

The Chx10 cDNA fragment containing 0.95 kb of the 3′ untranslated region (UTR) (StuI to 3′ end) and the 1.05 kb Chx10-1 cDNA fragment containing the 3′ UTR and the region encoding carboxyl terminal 39 amino acids were used for generating antisense riboprobes. Wholemount and section in situ hybridizations were performed as described (Riddle et al., 1993).

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References


