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Gene expression pattern

Expression of the zinc finger Egr1 gene during zebrafish embryonic development

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Abstract

Egr1 is a highly conserved zinc finger protein which plays important roles in many aspects of vertebrate development and in the adult. The cDNA coding for zebrafish Egr1 was obtained and its expression pattern was examined during zebrafish embryogenesis using whole-mount in situ hybridization. Egr1 mRNA is first detected in adaxial cells in the presomitic mesoderm between 11 and 20 h post-fertilization (hpf), spanning the 4–24 somite stages. Later, Egr1 expression is observed only in specific brain areas, starting at 21 hpf and subsequently increasing in distinct domains of the central nervous system, e.g. in the telencephalon, diencephalon and hypothalamus. Between 24 and 48 hpf, Egr1 is expressed in specific domains of the hypothalamus, mesencephalon, tegmentum, pharynx, retina, otic vesicle and heart.

Keywords: Egr1/Krox24; NGFI-A; Zebrafish; Expression pattern; Adaxial cell; Presomitic mesoderm; Telencephalon; Diencephalon; Hypothalamus; Mesencephalon; Tegmentum; Pharynx; Retina; Otic vesicle; Heart

1. Results and discussion

The Egr (early growth response) family of transcriptional regulator genes encode members of the Cys_2–His_2 class of zinc finger proteins. Egr1 encodes a 533 amino acid, 57 kDa protein containing three putative DNA-binding zinc finger sequences (Sukhatme et al., 1987; McMahon et al., 1990). Egr1 was originally identified as a rapid early response gene to a variety of proliferation stimuli, including NGF (Sukhatme et al., 1988), FGF and PDGF (Christy et al., 1988), and general serum proteins (Lemaire et al., 1988). During mouse embryogenesis, Egr1 mRNA is present in developing bone and muscle as well as several other mesodermal structures, including tooth mesenchyme and hair follicles (McMahon et al., 1990). In adult mouse tissues, Egr1 is restricted to heart, brain and lungs, and at lower levels to kidneys and spleen (Milbrandt, 1987; Sukhatme et al., 1988; Christy et al., 1988; Lemaire et al., 1988). This gene is also essential for differentiation in the monocyte/macrophage pathway (Krishnaraju et al., 1995; Nguyen et al., 1993). Studies of egr1-deficient mice revealed a defect in luteinizing hormone-β (LH-β) production (Topilko et al., 1998; Lee et al., 1996), and suggested its implication in prostate tumorigenesis (Abdulkadir et al., 2001).

The zebrafish homolog of the Egr1 gene was previously isolated and characterized (Drummond et al., 1994). We report here its particular expression pattern during zebrafish embryogenesis.

Egr1 mRNA was not observed during the first stages of development of the embryo until gastrulation (data not shown). Egr1 expression was first detected at the beginning of the segmentation period (Fig. 1). At the four somite stage, Egr1 mRNA is strongly expressed in the posterior adaxial cells of the segmental plate mesoderm, but is absent in the tailbud (Fig. 1A). Double in situ experiments revealed that Egr1 mRNA is present in posterior, myoD-expressing (Weinberg et al., 1996) adaxial cells just anterior to the tailbud (Fig. 1A). This expression pattern is maintained and restricted to adaxial cells and paraxial mesoderm during early somitogenesis (Fig. 1B–D). Double labelling with snail1 (Thisse et al., 1993) at the 12 somite stage confirmed the localization of Egr1 expression in posterior adaxial cells of the presomitic mesoderm (Fig. 1B). At the 23 somite stage, Egr1 expression is weaker and restricted to a small number of the most posterior adaxial cells (Fig. 1E,G); additional expression domains appear in the forebrain (Fig. 1E,F), more precisely in the telencephalon and hypothalamus, each in two symmetrical clusters of cells (Fig. 1F).

At 24 h post-fertilization (hpf), Egr1 expression continues in the ventral telencephalon and the hypothalamus (Fig. 2A). These expressions are maintained at 30 hpf, although at much

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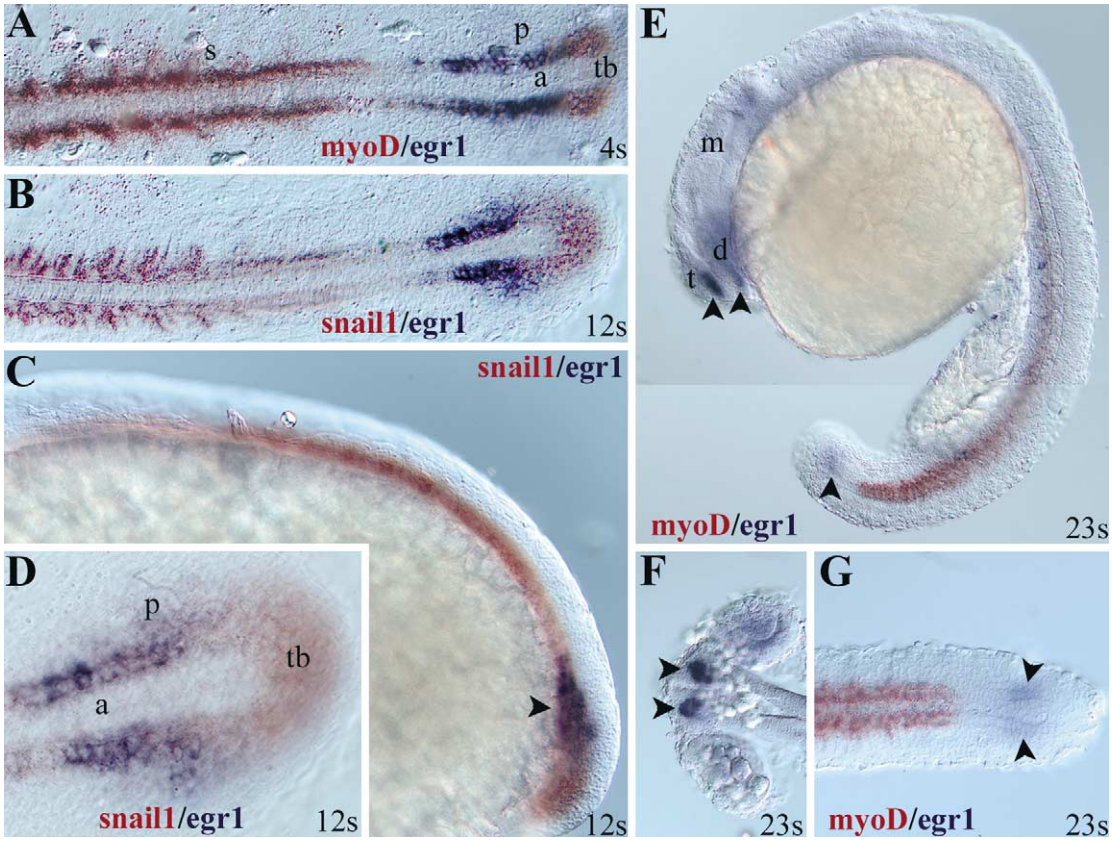

Fig. 1. Expression of Egr1 during the segmentation period. (A) Four somite stage, anterior to the left, dorsal view showing the expression of Egr1 (in blue) and myoD (in red). Egr1 is only present in the posterior adaxial cells of the segmental plate mesoderm. At 12 somite stage, anterior to the left, dorsal (B,D) and lateral (C) views, Egr1 mRNA (in blue) is again only present in adaxial cells of the posterior segmental plate mesoderm (C; arrowhead), adaxial cells also express snail1 (in red) (E–G). Twenty-three somite stage, anterior to the left, lateral (E) and dorsal (F,G) views showing expression of Egr1 (E–G; in blue) and myoD (E,G; in red). Egr1 is expressed in the telencephalon (E,F; arrowheads) and the diencephalon (E; arrowhead). Egr1 expression is weaker but maintained in the posterior adaxial cells of the segmental plate mesoderm (E,G; arrowheads). a, axial mesoderm; d, diencephalon; m, midbrain; p, paraxial mesoderm; s, somite; t, telencephalon; tb, tailbud.

lower levels in the hypothalamus. Additional expression is detected in the prospective pharynx and weakly in a group of cells at the ventral limit of the mesencephalon (Fig. 2B, black arrowheads). At 36 hpf, Egr1 mRNA is similarly present in the telencephalon, mesencephalon, pharynx and in the hypothalamus (Fig. 2C,G); an additional group of expressing cells appears at the posterior end of the hypothalamus, along the midline floor (Fig. 2C, black arrowheads).

Interestingly, new egr1 expression domains are observed at 40 hpf (Fig. 2D). In the brain, Egr1 mRNA is now also detected in the tegmentum and in the dorsal diencephalon (Fig. 2D, black arrowheads). The otic vesicle and the heart weakly express Egr1 mRNA (Fig. 2D, black arrowheads). Positive cells are also detected in the retina (Fig. 2H, black arrowheads). At 44 hpf, a clear expression is observed in the posterior part of the pharynx (Fig. 2E, black arrowhead).

Egr1 is strongly expressed in the forebrain and in the midbrain at 48 hpf (Fig. 2F,I); the signal is maintained in the pharynx and in the heart (Fig. 2F,J,K, black arrowheads), but is absent in the retina (Fig. 2F,I). Two adjacent sagittal sections were obtained at 48 hpf to confirm the expression domains (Fig. 2J,K). Strong expression is observed in the diencephalon, hypothalamus and at the anterior ventral end of the myelencephalon (Fig. 2F,J,K, black arrowheads).

2. Material and methods

Egr1 cDNA was obtained by reverse transcription–polymerase chain reaction on adult zebrafish brain RNA. We designed the primers (forward primer: 5′-ATGGCTG-CAGCCAAGACAGAG-3′ and reverse primer: 5′-TCAG-CAGATGTCGGCTGTCCG-3′) according to the exons of the Egr1 gene sequence (Drummond et al., 1994).

Whole-mount in situ hybridizations were performed as described (Hauptmann and Gerster, 1994). Sections of 30 μm were obtained on a vibratome as described (Bellefroid et al., 1996).

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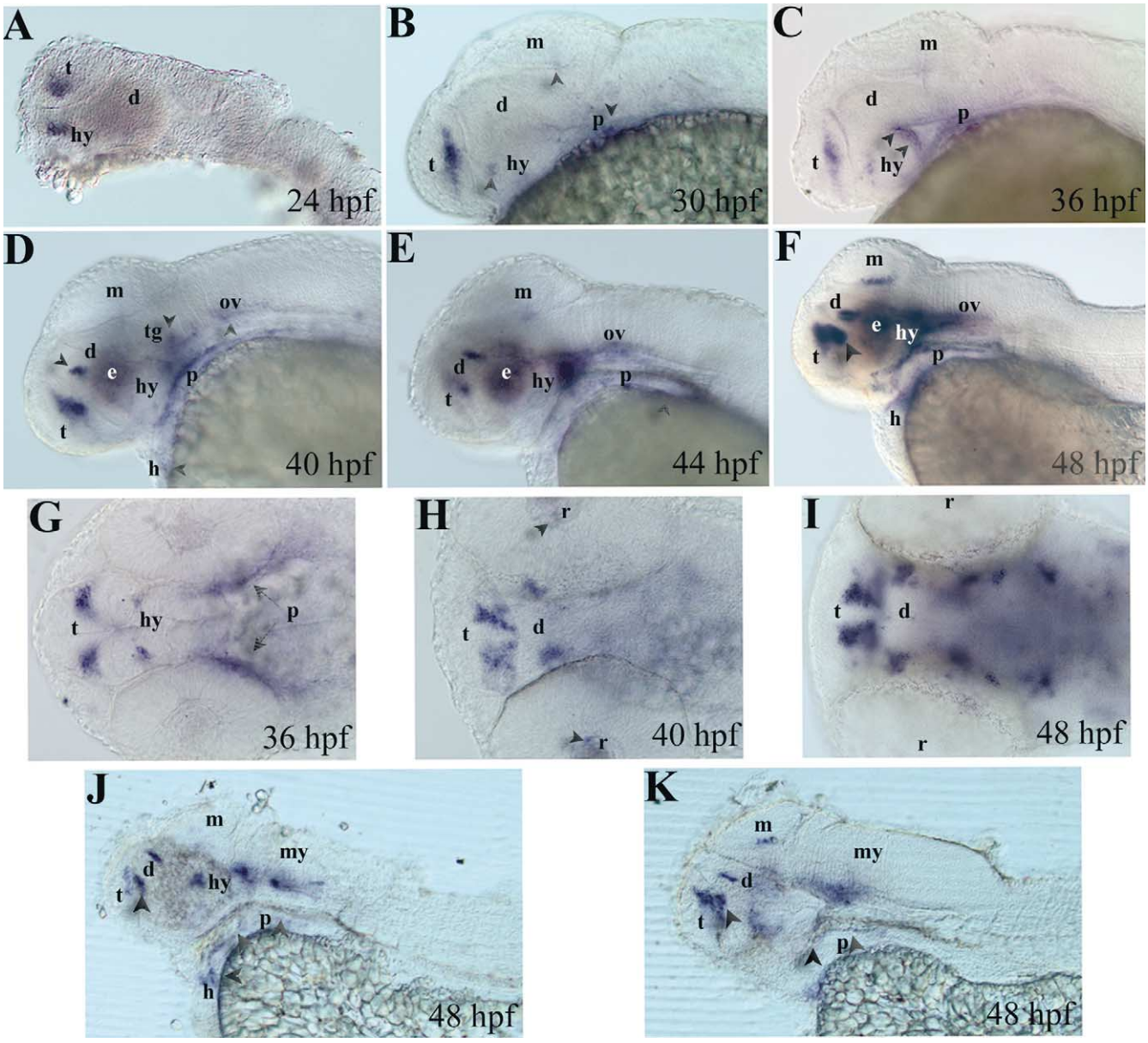

Fig. 2. Egr1 expression between 24 and 48 h post-fertilization (hpf): 24 (A), 30 (B), 36 (C,G), 40 (D,H), 44 (E) and 48 hpf (F,I–K). (A–F,J,K) lateral views, anterior to the left; (G–I) dorsal views, anterior to the left. 24 hpf (A), egr1 expression in the telencephalon and hypothalamus. Between 30 and 48 hpf (B–K), arrowheads point at new egr1 expression domains when compared to the preceding stage. (B) Thirty hours post-fertilization, expression in the mesencephalon and pharynx. (C,G) Thirty-six hours post-fertilization, signal detected along the midline floor of the hypothalamus. (D,H) Forty hours post-fertilization, arrowheads indicate new expression in the diencephalon, tegmentum, otic vesicle, heart and retina (H). (E) Forty-four hours post-fertilization, strong expression in the posterior pharynx. (F,I–K) Forty-eight hours post-fertilization, Egr1 is strongly expressed in the head, with a new expression in the diencephalon (F, black arrowhead). Egr1 signal is not observed in retina (I). (J,K) Two sagittal sections of the head region at 48 hpf showing overall head expression. Arrowheads point at the new expression in the diencephalon (J), pharynx (J,K) and heart (J). d, diencephalon; e, eye; h, heart; hy, hypothalamus; m, mesencephalon; my, myelencephalon; ov, otic vesicle; p, pharynx; r, retina; t, telencephalon; tg, tegmentum.

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