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Gene expression pattern

The heat-inducible zebrafish *hsp70* gene is expressed during normal lens development under non-stress conditions

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Abstract

In the present study, we show that the stress-inducible *hsp70* gene in zebrafish is strongly and specifically expressed during normal lens formation from 28 to 38 hours post-fertilization, and is subsequently downregulated by 2 days of age. Only weak constitutive *hsp70* mRNA signal was sporadically observed in other embryonic tissues. Similarly, transgenic fish carrying a 1.5 kb fragment of the *hsp70* promoter linked to eGFP exhibited fluorescence only in the lens. In contrast, both the endogenous *hsp70* gene and the transgene were strongly expressed throughout the embryo following heat shock at the same developmental stages.

Keywords: Zebrafish; Lens development; Heat shock proteins; hsp70; eGFP; Molecular chaperone; Eye

1. Results and discussion

Heat shock proteins (hsps) were initially identified as proteins expressed following exposure of cells to environmental stress, but several hsps were subsequently shown to play a critical role as molecular chaperones in normal intracellular protein folding and targeting events. Constitutively expressed hsps have usually been considered housekeeping proteins, yet a number of studies suggest that they also play more specific post-translational regulatory roles within developing and differentiating cells. For example, Hsp90 family members interact with and regulate the activity of a select number of kinases and transcription factors (Csermely et al., 1998), members of the Hsp70 family have recently been shown to be inhibitors of apoptosis (Mosser et al., 2000), and Hsp47 is a collagen chaperone essential for early mammalian development (Nagai et al., 2000). In zebrafish we have shown that stress-inducible members of the *hsp90* and *hsp47* gene families are also expressed during short temporal windows of somitic muscle and notochord development, respectively (Sass et al., 1996, 1999; Lele and Krone, 1997). Subsequent pharmacological inhibition experiments revealed that Hsp90 function is required for the differentiation of somitic muscle pioneer cells following their initial specification from somite progenitors (Lele et al., 1999). These data, together with the potential anti-apoptotic role of hsp70, prompted us to examine the early embryonic expression of the normally stress-inducible zebrafish *hsp70* gene (Lele et al., 1997).

Whole mount in situ hybridization analysis during the first 48 h of embryogenesis revealed a short temporal window of *hsp70* expression in the lens from approximately 28 to 42 hours post fertilization (hpf) in embryos raised at 28.5°C (Fig. 1). The eyes are readily identifiable by the presence of pigmented epithelium by 24 hpf, and while lens formation has begun by this stage of development (Li et al., 2000), *hsp70* expression was not yet detectable (Fig. 1B, C). Over the next 4 h, the eyes become more defined and *hsp70* transcripts were detected in the lens by 28 hpf (Fig. 1E, F). The lens expression of *hsp70* increased rapidly in intensity, and *hsp70* mRNA was abundant at 38 hpf (Fig. 1H, I, P). Expression was subsequently downregulated by 42 hpf (Fig. 1K, L) and was not discernable by 48 hpf (Fig. 1N, O, Q). Weak expression was detected only sporadically in non-lens cells of the embryo at comparable stages of development. In

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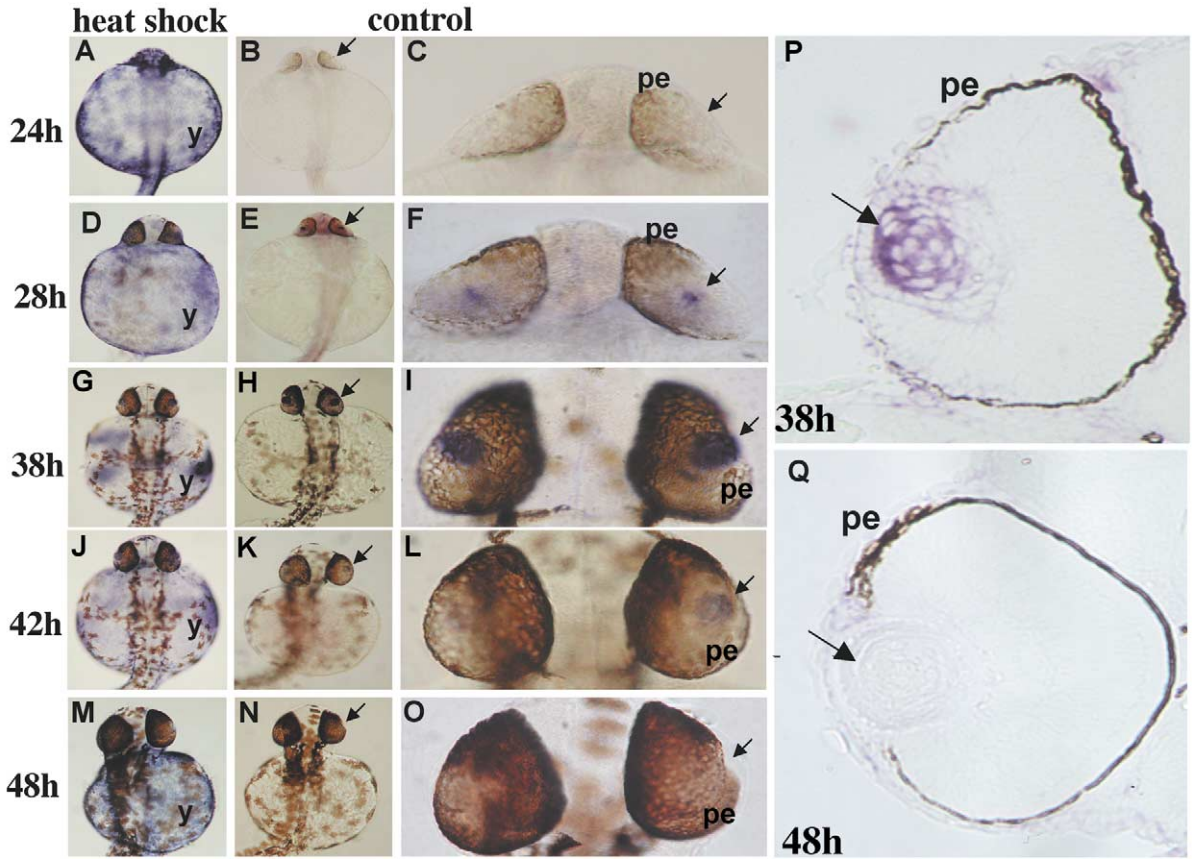

Fig. 1. Whole mount in situ hybridization analysis of *hsp70* mRNA. Anterior is to the top for all figures. Dorsal/ventral views of entire embryo and enlargement of the anterior region, respectively, are shown for 24 hpf (A,B,C), 28 hfp (D,E,F), 38 hfp (G,H,I), 42 hpf (J,K,L), and 48 hpf (M,N,O). Sections of the eye are shown in panels P,Q. Constitutive *hsp70* mRNA is first detectable in embryos raised at 28.5°C as weak expression in the lens at 28 hpf (black arrows in (E,F)). Peak levels of *hsp70* mRNA are observed at 38 hpf (H,I,P) with significant downregulation evident by 42 hfp (K,L) and no signal present by 48 hpf (N,O,Q). pe, Pigmented epithelium; y, yolk.

contrast, the *hsp70* gene was expressed throughout the embryo following 1 h heat shock at 37°C (Fig. 1A, D, G, J, M). Additionally, GFP fluorescence in a strain of zebrafish carrying 1.5 kb of the *hsp70* promoter driving expression of an eGFP reporter gene (Halloran et al., 2000) was also observed specifically in the lens under non-stress conditions (Fig. 2A), whereas expression was detectable throughout the embryo following heat shock (Fig. 2B).

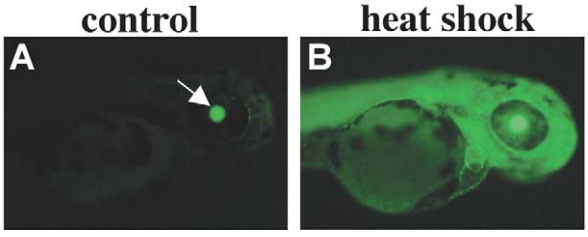

Fig. 2. Expression of *hsp70*-eGFP reporter gene in live transgenic embryos. The *hsp70*-eGFP reporter gene is constitutively expressed in the lens under non-stress conditions and the stable eGFP gene product is shown in an embryo at 72 hpf (white arrow in (A)). In contrast, a typical stress-induction of the reporter is shown at the same developmental stage following a 1 h heat shock (B).

While zebrafish cultured cells and embryos raised at 28.5°C do not exhibit a discernable heat shock response (Krone et al., 1997), it was possible that developing lens fibres are more sensitive to temperature stress than other cell types. However, embryos raised at the suboptimal growth temperature of 25°C, while developing much slower, also exhibited lens-specific *hsp70* expression during the same relative window of lens formation (data not shown).

The data presented here show that the zebrafish *hsp70* gene is strongly and specifically expressed during formation of the embryonic lens, and expression is directed to the lens by sequences within the *hsp70* promoter. This may be more widespread in vertebrates, since chicken *hsp70* mRNA has been detected in cultured lens explants using RNA blot analysis (Dash et al., 1994). However, it is not known if expression is limited exclusively to the lens, and whether there is a specific window of chicken eye development when expression occurs.

2. Material and methods

Embryos were collected, maintained at 28.5°C, and

staged according to Kimmel et al. (1995) and Westerfield et al. (1995). Heat shock treatments were carried out for 1 h at 37°C. Digoxigenin-labeled antisense riboprobe was synthesized from the *hsp70–4* PCR fragment previously cloned by Lele et al. (1997), and in situ hybridization was performed according to Jowett (1997) with modifications. Both DIC and fluorescence imaging were carried out on a Nikon E-600 microscope equipped with a Nikon Coolpix digital camera.

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