Gene Expression Patterns 11 (2011) 360–369

Contents lists available at ScienceDirect

Gene Expression Patterns

journal homepage: www.elsevier.com/locate/gep

Identification and expression of *soul/p22HBP* genes in zebrafish

Antonio Emidio Fortunato [a,b,c,e,1], Fernanda Langellotto [a,d,1], Paolo Sordino [a,*]

[a] Stazione Zoologica Anton Dohrn, Laboratory of Cellular and Developmental Biology, 80121 Napoli, Italy
[b] UPMC Univ. Paris06, UMR7238, Génomique des Microorganismes, 15 rue de l'École de Médecine, 75006 Paris, France
[c] CNRS, UMR7238, Génomique des Microorganismes, Paris, France
[d] University of Massachusetts Medical School (UMMS), Department of Ophthalmology & Gene Therapy Center, 01606 Worcester, MA, USA
[e] Università degli studi di Palermo, Dipartimento di Biologia Cellulare e dello Sviluppo, 90128 Palermo, Italy

ARTICLE INFO

Article history:
Received 12 November 2010
Received in revised form 17 March 2011
Accepted 22 March 2011
Available online 2 April 2011

Keywords:
Heme binding protein
Iron
Tetrapyrrole metabolism
Embryonic development
SOUL/p22HBP family
Pineal gland
Yolk syncytial layer
Pronephros

ABSTRACT

The SOUL/p22HBP family is an evolutionarily ancient group of heme binding proteins with a main function as cytosolic buffer against tetrapyrrole accumulation. Structural and biochemical evidence suggest specialized roles in blood formation, necrotic cell death and chemotaxis. To date, nothing is known about the precise activity and expression patterns of this class of heme binding proteins during development. The zebrafish genome possesses five *soul* genes belonging to two subgroups, and no *p22HBP* orthologous gene. Here, spatial and temporal expression patterns are reported for zebrafish *soul1*, *soul2* and *soul4* genes. All three *soul* genes are maternally transcribed, and their zygotic expression takes place in unique (heart, pharynx, yolk syncytial layer, brain, eyes, lateral line) and overlapping (pronephros, pituitary gland, olfactory and otic vesicle) regions of the zebrafish embryo. Our study constitutes the first detailed analysis of *soul* gene expression in metazoan development, and provides the basis to understand the genetics of tetrapyrrole metabolism in a wide range of embryonic processes.

1. Results and discussion

The *soul/p22HBP* genes encode for heme binding proteins and have been originally identified in mouse and humans (p22HBP) (Taketani et al., 1998) and in chick (SOUL) (Zylka and Reppert, 1999). The SOUL/p22HBP proteins share several aspects including amino acid sequence similarity (around 48%), size (22–28 kDa), tertiary structure, tetrapyrrole binding and cytosolic distribution (Sato et al., 2004; Taketani et al., 1998; Zylka and Reppert, 1999). Tetrapyrrole affinity K_d (dissociation constant) value and cellular localization imply that SOUL/p22HBP proteins are representative of porphyrin metabolism operating as intracellular buffer, a physiological function that is accomplished through diverse structural and chemical properties (Blackmon et al., 2002; Dias et al., 2006; Micaelo et al., 2010; Sato et al., 2004; Taketani et al., 1998). Murine p22HBP is a monomer featuring a novel eukaryotic heme binding surface similar to that of bacterial transcription factors involved in drug resistance and detoxification mechanisms (Dias et al., 2006; Gell et al., 2006). In mammals, the free dimeric state of SOUL apo-form is induced into a hexamer upon heme binding by a mechanism involving a histidine (His42) that is missing in p22HBP (Sato et al., 2004).

A variety of biological functions have been proposed for the SOUL/p22HBP proteins. Reports on gene expression in eye, pineal gland, liver and kidney suggest distinct functions in circadian rhythms, anti-inflammatory response, cell differentiation and necrosis (Babusiak et al., 2005; Bohn and Winckler, 1991; Natt et al., 2009; Taketani et al., 1998; Zylka and Reppert, 1999). First, consistent with a role in blood differentiation, the *p22HBP* gene is up-regulated by GATA-1, a blood-specific transcription factor (Welch et al., 2004), while p22HBP promotes heme biosynthesis and hemoglobin assembly in Mouse Erythroleukemia cells (Babusiak et al., 2005; Taketani et al., 1998). Second, release of the formylpeptide receptor-like 2 ligand (F2L peptide) by N-terminal cleavage of HEBP1, the human p22HBP ortholog, exerts anti-inflammatory and chemoattractive functions (Gao et al., 2007; Lee et al., 2008). Third, the interaction between HEBP2, the human SOUL ortholog, and the Leucine-rich PPR motif-containing protein suggests also functions associated with cell membrane, cytoskeleton and nucleus (Liu and McKeehan, 2002). Fourth, the hypothesis of an implication in cell death is based on the evidence that HEBP2 induces necrosis in NH3T3 cells through the specific action of its BH3 domain, a well-known cell death-related motif (Szigeti et al., 2006, 2010). Moreover, human SOUL interacts with Programmed

* Corresponding author. Tel.: +39 081 5833283; fax: +39 081 7641355.
E-mail addresses: antonio.fortunato@upmc.fr (A.E. Fortunato), fernanda.langellotto@gmail.com (F. Langellotto), sordino@szn.it, paolo.sordino@szn.it (P. Sordino).
[1] These authors contributed equally to this work.

1567-133X/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.gep.2011.03.006

Cell Death 6, a Ca^{2+}-binding protein tightly associated with apoptotic processes (Krebs et al., 2002; Rual et al., 2005).

Homologs of the *soul*/*p22HBP* family have been identified in plants (*Arabidopsis thaliana*; Takahashi et al., 2008) and single-celled algae (*Chlamydomonas reinhardtii*; Wagner et al., 2006, 2008), in relation to light-mediated responses (Khanna et al., 2006), as well as to chloroplast and vacuole functions (Jaquinod et al., 2007a,b; Peltier et al., 2006, 2004). Interestingly, *Arabidopsis* SOUL/p22HBP proteins are closer to p22HBP in terms of heme binding surface and absence of mammalian SOUL-specific His42 (Takahashi et al., 2008).

With the aim to elucidate the expression profiles of the *soul*/*p22HBP* gene family during embryonic development, we have analyzed the expression of three zebrafish *soul* orthologs by means of whole-mount *in situ* hybridization (WISH) and real-time quantitative PCR (qPCR). Low accuracy in the prediction of gene coding sequences among genome releases, and uncertain WISH results have delayed the analysis of the *soul3* and *soul5* genes. In order to avoid redundancy and to show only representative data, not all developmental stages presented in qPCR have correspondence with WISH stages.

1.1. Zebrafish SOUL family bioinformatics analysis

Five *soul* genes were found in the zebrafish genome (Zv5–Zv9 releases, www.ensembl.org) by using the murine p22HBP and the avian SOUL sequences as queries. The affiliation to the SOUL/p22HBP family was confirmed by analysis of sequence alignment and predicted functional domains in the Prosite protein database. These genes were named following the order of identification: *soul1* (ENSDARG00000042630), *soul2* (ENSDARG00000039499), *soul3* (ENSDARG00000063452), *soul4* (ENSDART00000115108) and *soul5* (ENSDARG00000075015). Three of them are placed on zebrafish chromosomes 3, 13 and 20 (*soul5*, *soul2* and *soul1*, respectively), while two are embedded in genomic scaffolds (*soul3* and *soul4*, Zv8_NA2449: 14,812–23,700 and Zv8_scaffold2991: 75,012–86,155, respectively) due to incomplete genome assembly. Intraspecies sequence similarity of fish, avian and mammalian SOUL proteins is constantly around 48%, suggesting high rates of molecular divergence among *soul* genes. At interspecies level, SOUL sequence comparison between fish and higher vertebrates ranges from 41% to 60% (Fig. 1).

Bioinformatics analyses performed with Phobius and SignalP3.0 servers predicted a signal peptide involved in protein secretion in SOUL2, with a cleavage site around residue 25. Interestingly, a cysteine at position 30, near the cleavage site of a signal peptide, could represent a *S*-acylation site, a common feature among membrane-associated proteins (Linder and Deschenes, 2003). An N-terminal signal peptide for protein secretion was predicted also in SOUL4, with cleavage site around residue 20 (Fig. 1). Differential distribution of signal peptides and membrane binding residues among zebrafish SOULs are suggestive of cell compartment specificity for some of these proteins. Beyond the presence of the SOUL domain (pfam: PF04832), no additional protein domain could be identified through bioinformatics tools. Similarly, sequence alignment of zebrafish SOUL proteins does not allow clear identification of the p22HBP-specific F2L and of the mammalian SOUL-specific BH3 domains (Lomonosova and Chinnadurai, 2008; Szigeti et al., 2010). The His42 residue is present only in SOUL3 and SOUL5, two proteins that share 47% and 53% sequence identity with murine SOUL, respectively.

Based on sequence alignment and similarity, two types of orthologous SOUL proteins were recognized previously in the literature since Zylka and Reppert (1999). Interestingly, our phylogenetic analysis of vertebrate SOUL proteins revealed three different clades (Fig. 2), one with mammalian and three zebrafish SOUL clustering with chick SOUL2, one consisting of chick and mammalian p22HBPs, and one with the prototypic chicken SOUL grouped with two zebrafish SOUL proteins. Tree topology supports an ancient divergence between the avian and mammalian SOUL, with p22HBP either duplicated after fish divergence, or secondarily lost in teleosts. The zebrafish *soul*/*p22HBP* gene family was likely shaped by the three rounds of whole-genome duplication (WGD) that occurred before bony fish evolution, with lineage-specific loss of *p22HBP* orthologs (Amores et al., 1998). A comprehensive analysis of SOUL/p22HBP molecular phylogeny is required to understand the evolutionary history of this poorly known family of heme binding proteins.

1.2. soul1 gene expression

To elucidate the extent to which SOUL/p22HBP factors participate to embryogenesis, we examined the expression of three *soul* genes in the zebrafish. Initially, a maternal contribution of *soul1* transcript was detected by qPCR and WISH, with uniform distribution during the early cleavage period (1.25 h post-fertilization, hpf) (Figs. 3A and 4A). From late gastrula, *soul1* expression became quantitatively low, with small peaks of zygotic transcription at 36 and 60 hpf (Fig. 3). Although this peak was concomitant with the definitive hematopoietic wave occurring in the pronephros (Hsia and Zon, 2005), no *soul1* expression was detected in erythroid precursors at any analyzed stage. At the onset of somitogenesis (11 hpf), *soul1* transcript was detectable only in the yolk syncytial layer (YSL) underlying the embryo (Fig. 4B), where its distribution was later restricted to the emerging yolk extension (YE) (Fig. 4C, D and D′). During development, this YE-specific domain first extended anteriorly, then it was restricted posteriorly and finally disappeared by 60 hpf (data not shown). From 40 hpf, *soul1* transcript was also detected in the YSL that covers the yolk sac (Fig. 4E). The identification of SOUL protein (HEBP2 or Placental Protein 23) in the human placenta suggests that a relationship between *Soul* genes expression and extra-embryonic tissues with trophic functions is a basal feature of vertebrate evolution (Bohn and Winckler, 1991). Also other genes involved in iron and heme biosynthesis, such as *fpn1* (*ferroportin1*) and *cp* (*ceruloplasmin*), are expressed in the YSL (Donovan et al., 2005; Korzh et al., 2001). This observation lends support to SOUL1 playing a role in iron and heme/tetrapyrrole synthesis and/or trafficking during embryogenesis. *soul1* expression took place in olfactory vesicles (Fig. 4E and F) and pituitary gland (Fig. 4E, E′ and F′) between 40 and 48 hpf, and in otic vesicles and heart (Fig. 4G and G′) at 60 hpf. Even though *soul1* was expressed in the pituitary gland at a time when thyrotrope and somatotrope cell types undergo terminal differentiation, it is difficult to speculate about specific function(s) in the development of this neuroendocrine organ (Pogoda and Hammerschmidt, 2009).

1.3. soul2 gene expression

While the maternal transcript of the *soul2* gene was lowly represented, a relatively strong transcriptional level was observed throughout zygotic development, with a peak at 48 hpf, when the embryonic kidney begins blood filtration (Fig. 3B). WISH analysis revealed that, similar to *soul1*, *soul2* is maternally expressed in the whole embryo from the early cleavage stage (Fig. 5A). Later, zygotic expression during early somitogenesis was localized in the ventral portion of the entire embryo (Fig. 5B).

At 18 hpf, *soul2* transcription was initially detected in the cloaca, progressively extending distally across the forming kidney (Fig. 4C, D and F–H). Following Wingert et al's. (2007) pronephros segmentation map according to somite boundaries, *soul2* expression incorporated the distal late segment of the pronephros,