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Upstream stimulatory factors, USF1 and USF2 are differentially expressed during *Xenopus* embryonic development

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ABSTRACT

Upstream stimulatory factors (USF) 1 and 2 are members of the basic helix–loop–helix leucine zipper transcription factor family. They are considered to play critical roles in cell-cycle regulation and chromatin remodeling. Their gene expression patterns are considered ubiquitous but have not been fully investigated in terms of embryogenesis. We examined the expression of the genes encoding USF1 and USF2 in *Xenopus laevis* during embryonic development. Expression of both genes was first detected as maternal transcripts and was observed continuously throughout development. However, *in situ* hybridization analysis revealed that the two genes were expressed differentially. In the late blastula, both genes were expressed in the blastocoel roof and marginal zone. At the gastrula stage, *USF2* was strongly expressed in the sensorial layer of the ectoderm and in the mesoderm, whereas *USF1* expression was hardly detectable. From the neurula stage onward, expression of both genes was markedly enhanced in the neural tissues, neural crest, eye and otic vesicle. However, spatial expression of the genes within the neural tube differed in that the strongest *USF1* signals were observed in the lateral region of the basal plate and the strongest *USF2* ones in the dorsal region of the neural tube. Expression of the two genes occurred in different mesoderm derivatives at the tailbud stage (*USF1*, somite; *USF2*, pronephros and lateral plate mesoderm of the tail region). *USF1* was expressed in the notochord of the early neurula, but was lost at the stage.

Upstream stimulatory factor (USF) family proteins are well known as ubiquitously expressed transcription factors (Gregor et al., 1990; Sirito et al., 1994; reviewed by Corre and Galibert, 2005). In vertebrates, there are two types of protein, USF1 and USF2, which share the conserved basic helix–loop–helix leucine zipper (b-HLH-LZ) domains. These two factors form a homo- or heterodimer via their HLH-LZ domains. They bind to the E-box consensus site (CANNTG) and pyrimidine-rich initiator (inr) element adjacent to the start site in the TATA-less promoter. USFs interact with various factors (tissue-specific or general transcription factors, chromatin remodeling enzymes, and preinitiation complexes of general transcription factors). Functional analysis in cultured mammalian cells has indicated that USFs are involved in the gene networks of stress responses, the cell-cycle, and cell proliferation (reviewed by Corre and Galibert, 2005).

Gene-targeting studies have revealed the roles of *USF* genes at an individual level. *USF1/USF2* compound mutant mice show embryonic lethality, and *USF2* null-mutant mice have a smaller than normal mice, and displayed proportionate body features (Sirito et al., 1998). *USF1* null mice lack obvious morphological abnormalities, but they occasionally have epileptic seizures and show enhanced barbering behavior (Sirito et al., 1998). These results indicate that the *USF* genes are important for embryonic development and brain function. However, there have been few in-depth analyses of their role in vertebrate embryonic development, and especially in neural development.

Although expression of the genes encoding USF1 and USF2 is ubiquitous, USF homodimers and heterodimers are found in different ratios in different cell types (Sirito et al., 1994; Viollet et al., 1996). It has been proposed that these factors may control differently target genes by specific interactions via their distinct N-terminal domains with different transcription factors (Corre and Galibert, 2005). Functional differences between USF1 and USF2 may partly underlie the phenotypic differences in the mutant mice. However, clarification of the comparative expression profiles of the two *USF* genes is necessary for us to understand their biological significances more clearly.

We examined the expression profiles of *Xenopus laevis USF1* and *USF2* in the context of embryonic development. We expected that the analysis of *Xenopus* embryos would give us a clear picture of early embryonic expression patterns. Kaulen et al. (1991) earlier reported the USF1 as a B1 factor, TFIIIA promoter binding protein. However, the expression profile of the gene encoding B1 has not been reported.

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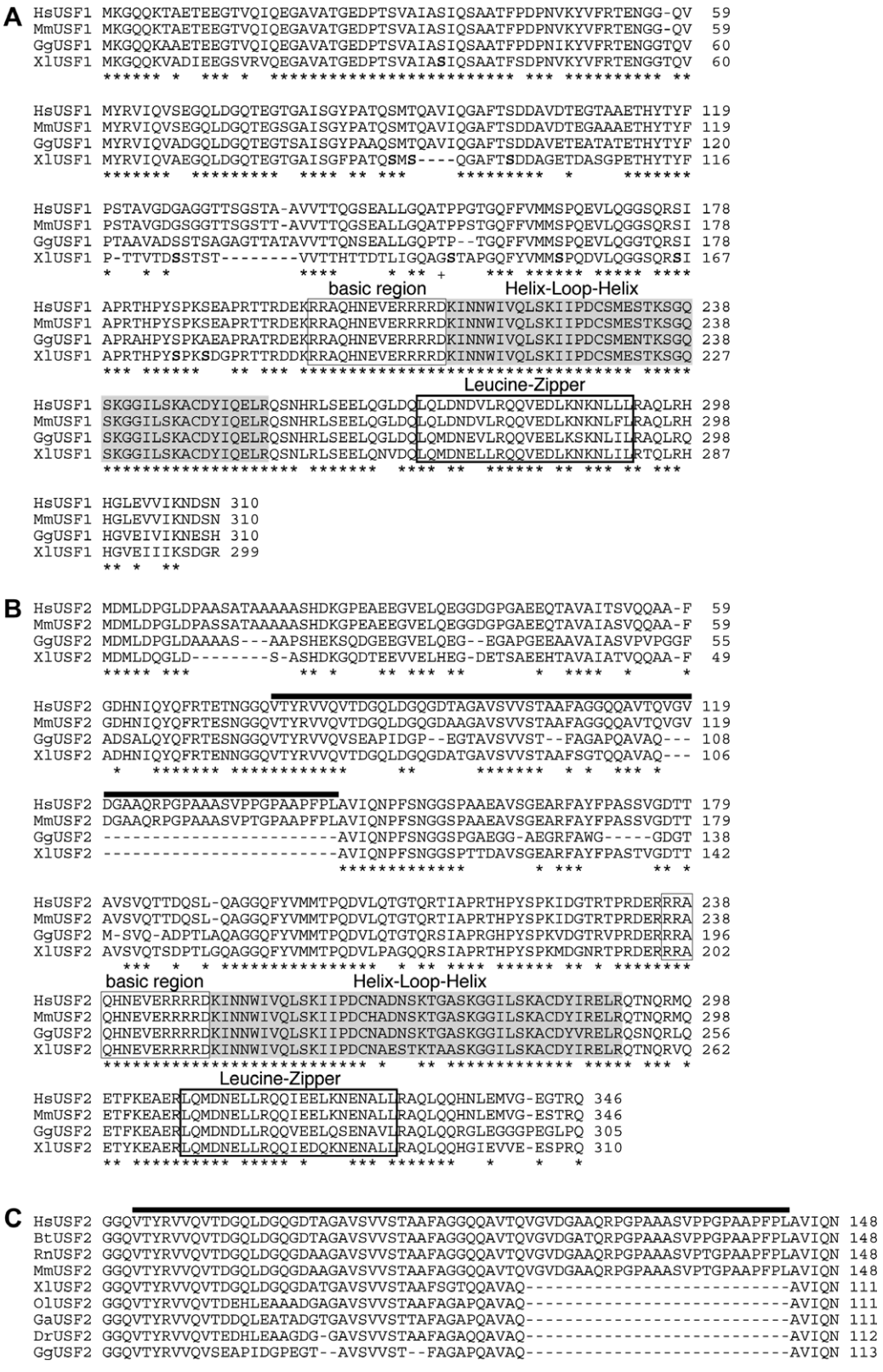

Fig. 1. Amino acid sequence comparisons of USF proteins. Sequence alignments of (A) USF1, entire region; (B) USF2, entire region and (C) a USF2 region including exon 4 of the human sequence. Amino acid sequences of USF proteins (Hs, human; Mm, mouse; Gg, chicken; Xl, frog; Rn, rat; Bt, cow; Ol, medaka fish; Dr, zebrafish; Ga, stickleback fish) were aligned by CLUSTALX (1.8) with default parameters (Jeanmougin et al., 1998) and optimized manually. Highly conserved regions characteristic of USF proteins are indicated by boxes (as labeled on the sequences). Residues shown by bold letter in *Xenopus laevis* USF1 sequence are possible MAP kinase-phosphorylation targets, which are predicted by KinasePhos (2.0) (Wong et al., 2007). Bold rules above sequences in B and C indicate the region corresponding to the human *USF2* fourth exon. Asterisks indicate amino acid residues conserved among all species. The position of T153 in human USF1 is indicated by +. USF1 sequences were derived from BAA76541 (human); CAA64627 (mouse); AAV91517 (chicken) and AAH97655 (frog). USF2 sequences were derived from CAA68942 (human); AAB60674 (mouse); AAT27442 (chicken); AAH87339 (frog); AAI28736 (rat); NP_001001162 (cow); ENSORLP00000011299 (medaka); CAK05404 (zebrafish). The amino acid sequence of stickleback USF2 was deduced from the nucleotide sequence data of BT028064 (*Gasterosteus aculeatus* clone CNB117-F09 mRNA sequence).