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Divergence in regulation of the PEA3 family of ETS transcription factors

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Abstract

Here, we report the cloning of a cDNA encoding zebrafish ER81, a member of the PEA3 family of Ets transcription factors. Strikingly, the spatial and temporal expression of *er81* is significantly different from its *Xenopus* orthologue, XER81, whose expression is more reminiscent of the FGF dependant zebrafish PEA3 family members. In keeping with this observation, while *pea3*, *erm* and XER81 require FGF activity for their expression, *er81* does not require FGF signalling. Our results suggest that, since the vertebrate specific expansion of the PEA3 subfamily of Ets transcription factors, the regulation of PEA3 genes has been independently modified during the evolution of different vertebrate lineages.

Keywords: Zebrafish; PEA3 family; *er81*; FGF; Evolution

1. Results and discussion

Members of the Fibroblast Growth Factor (FGF) family of secreted proteins play crucial roles in patterning the vertebrate embryo. To gain a better understanding of the role of FGF signalling during development, the transcriptional responses elicited by FGFs have been studied in a variety of model systems. For example, genes have been identified whose expression pattern is similar to that of *fgf8* during early zebrafish development, the so-called *fgf8* syn-expression group, including two Ets transcription factors of the PEA3 subgroup, *pea3* and *erm* (Münchberg et al., 1999; Raible and Brand, 2001; Roehl and Nusslein-Volhard, 2001). The PEA3 family contains a third member, Er81. However, while expression of the *Xenopus* gene, XER81, is stimulated by FGFs, no similar FGF/ER81 transcriptional relationship has been documented in other species (Chen et al., 1999; Münchberg and Steinbeisser, 1999). The three members of the PEA3 family represent a vertebrate specific expansion of a single ancestral Ets gene (Laudet et al., 1993, 1999). One explanation for potential changes in FGF regulation within this family could be that, after the vertebrate specific expansion in the PEA3 gene family, FGF regulation of *Er81* was lost in higher vertebrates but retained in lower vertebrates. This possibility remains to be addressed, however, as although expression of zebrafish *pea3* and *erm* has been shown to be FGF responsive, a zebrafish *er81* gene has not yet been identified (Raible and Brand, 2001; Roehl and Nusslein-Volhard, 2001).

1.1. Cloning and characterisation of er81

Our interest in the evolution of the regulation of gene expression between higher and lower vertebrates lead us to search for zebrafish *er81* orthologues. An RT-PCR strategy was designed to isolate a cDNA corresponding to a Er81-like Ets domain containing transcription factor. The resulting cDNA codes for a putative protein of 476 amino acids with strong homology to ER81 proteins along the entire length of the predicted coding region (Fig. 1A). Screening of the zebrafish databases revealed a second Est encoding a PEA3 family member distinct from Pea3

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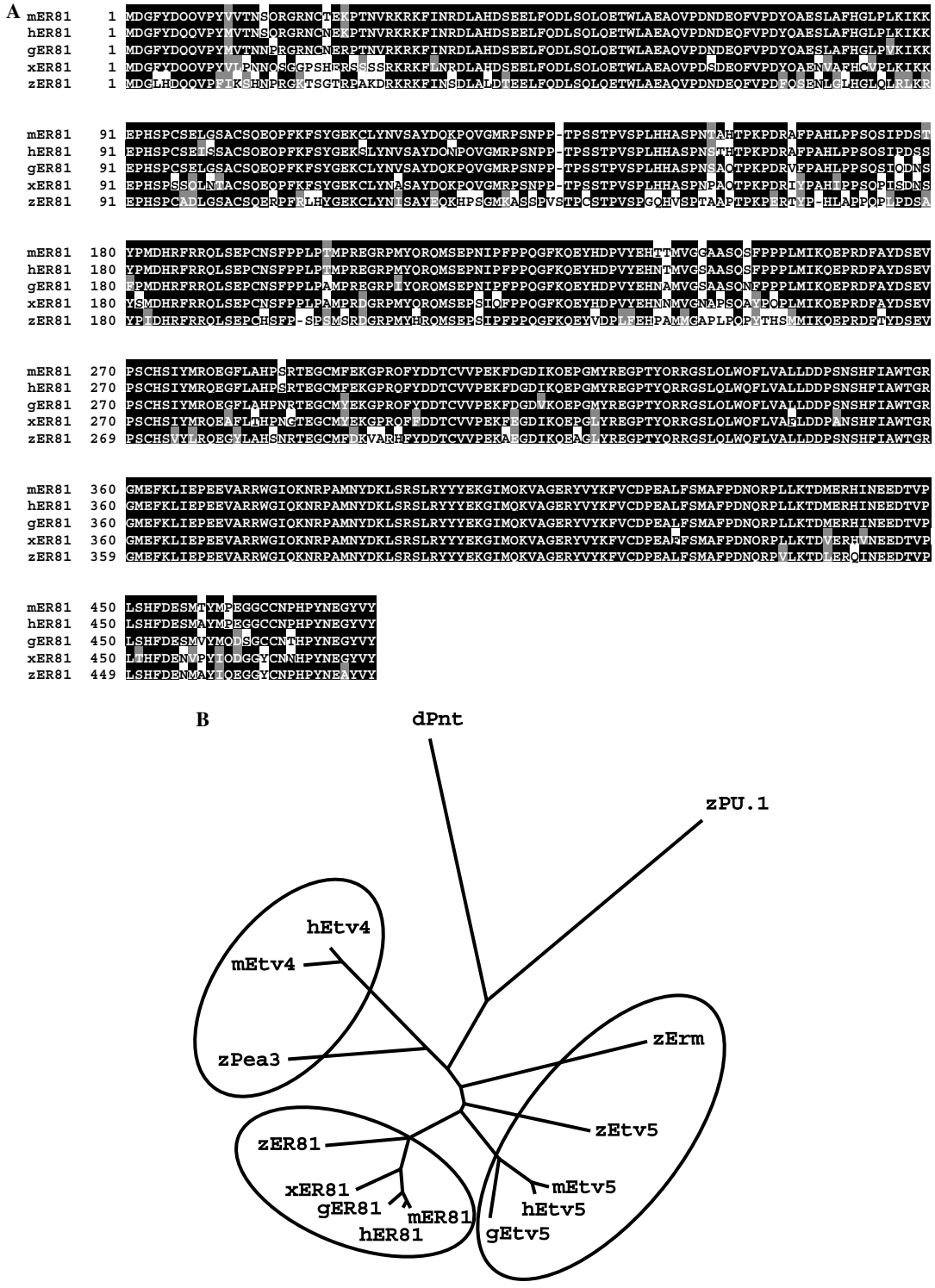

Fig. 1. (A) Multiple amino acid sequence alignment of zebrafish ER81 (zER81) with its human (hER81), mouse (mER81), chicken (gER81), and *Xenopus* (xER81) orthologues. Conserved amino acids are highlighted; black boxes indicate identical residues whereas grey boxes show similar residues. Dashed lines represents gaps introduced to align sequences. (B) Phylogenetic tree obtained with all known full length members of the PEA3 subfamily of Ets domain transcription factors. The amino acid sequence of Pnt, a *Drosophila* Ets transcription factor, and zPU.1, a zebrafish Ets transcription factor from a different subfamily, have been included as outgroups in the analysis.

and Erm, but also from the Er81 described above. This second clone encodes a full length Ets transcription factor and has been annotated as zebrafish *ets variant gene 5* or *etv5* (Kudoh et al., 2001). As in other species *erm* and *etv5* are interchangeable names for the same gene, it appears that, as for many genes, ray-finned fish possesses two duplicates of an ancestral *etv5*/*erm* gene (Taylor et al., 2003). The relationship between the four zebrafish PEA3 family members