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RNA helicase *Ddx39* is expressed in the developing central nervous system, limb, otic vesicle, branchial arches and facial mesenchyme of *Xenopus laevis*

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ABSTRACT

Ddx39, a DEAD-box RNA helicase, is a part of the homeostatic machinery that regulates the switch between cellular proliferation and differentiation. *Ddx39* was shown to be differentially regulated in *Xenopus laevis* using a differential screen of mRNAs from regenerating limbs (King et al., 2003). Here, the expression patterns of *Ddx39* in developing limb and nervous system are reported. *Ddx39* was detected by RT-PCR in the *Xenopus* embryo, the earliest stage examined. Localization of the message by whole-mount *in situ* hybridization at stage 17 showed it to be localized primarily to the developing nervous system. *Ddx39* was present in the ventricular region of the developing neural tube up to and including stage 48, and was also localized to the head mesenchyme, pharyngeal arches, and paraxial mesoderm. Strong label was also present in the developing limb buds at stages 48–55. Analysis of expression patterns in cryosections of the developing eye at stage 38 and 47 showed *Ddx39* in the ciliary marginal zone (CMZ) adjacent to the neural retina and within the lens epithelium. *Ddx39* was also present in the anterior eye during fibroblast growth factor 2 (FGF2)-mediated retinal regeneration. BrDU incorporation analyses and double-label studies with proliferating cell nuclear antigen showed that *Ddx39* message was restricted to a subpopulation of proliferating cells in the developing and regenerating optic cup.

Embryonic, mature, and regenerating nervous systems require a fine balance between proliferation and differentiation (Xia et al., 2006). The retina of *Xenopus laevis* (African clawed frog) is a powerful model system for studying the mechanisms that regulate this balance. The progenitor cells that give rise to the embryonic retina are derived from the diencephalic neural tube which undergoes morphogenetic movements to give rise to the optic cup (Dorsky et al., 1995; Harris and Perron, 1998; Perron et al., 1998; Wang and Harris, 2005; Cayouette et al., 2006). In addition to the embryonic retinal progenitors, the mature *Xenopus* retina also contains a region adjacent to and continuous with the neural retina, known as the ciliary marginal zone (CMZ). The CMZ contains small population of stem cells that produce highly proliferative progenitor cells capable of maintaining growth of the amphibian eye in maturity (Harris and Perron, 1998; Perron et al., 1998; Reh and Fischer, 2006). Finally, the retinal pigmented epithelium (RPE) is capable of undergoing a process known as transdifferentiation in the presence of FGF2, which will give rise to a proliferating neuroepithelium that will regenerate the retina (Sakaguchi et al., 1997; Araki, 2007; Yoshii et al., 2007; Vergara and Del Rio-Tsonis, 2009).

Members of many signaling pathways have been studied with regard to maintenance of proliferation or differentiation; however the epigenetic mechanisms by which large scale changes in the states of the cell are not understood. One group of proteins that hold promise for increased understanding in this area includes RNA helicases. RNA helicases are enzymes that bind and separate double-stranded RNA and/or displace RNP complexes in a nucleotide triphosphate-dependent manner (Matson and Kaiser-Rogers, 1990; Pyle, 2008). The RNA helicases are grouped into five categories, superfamily 1–3 (SF1–3) and families 4 and 5, based on sequence and motif conservation (Gorbalenya and Koonin, 1991). SF2 is comprised of the RNA helicases that contain a motif with the amino acid sequences D-E-A-D, termed the DEAD-box proteins, or related DEAH, DExH, and DExD proteins (Rocak and Linder, 2004; Li et al., 2008). Two members of SF2 have been implicated

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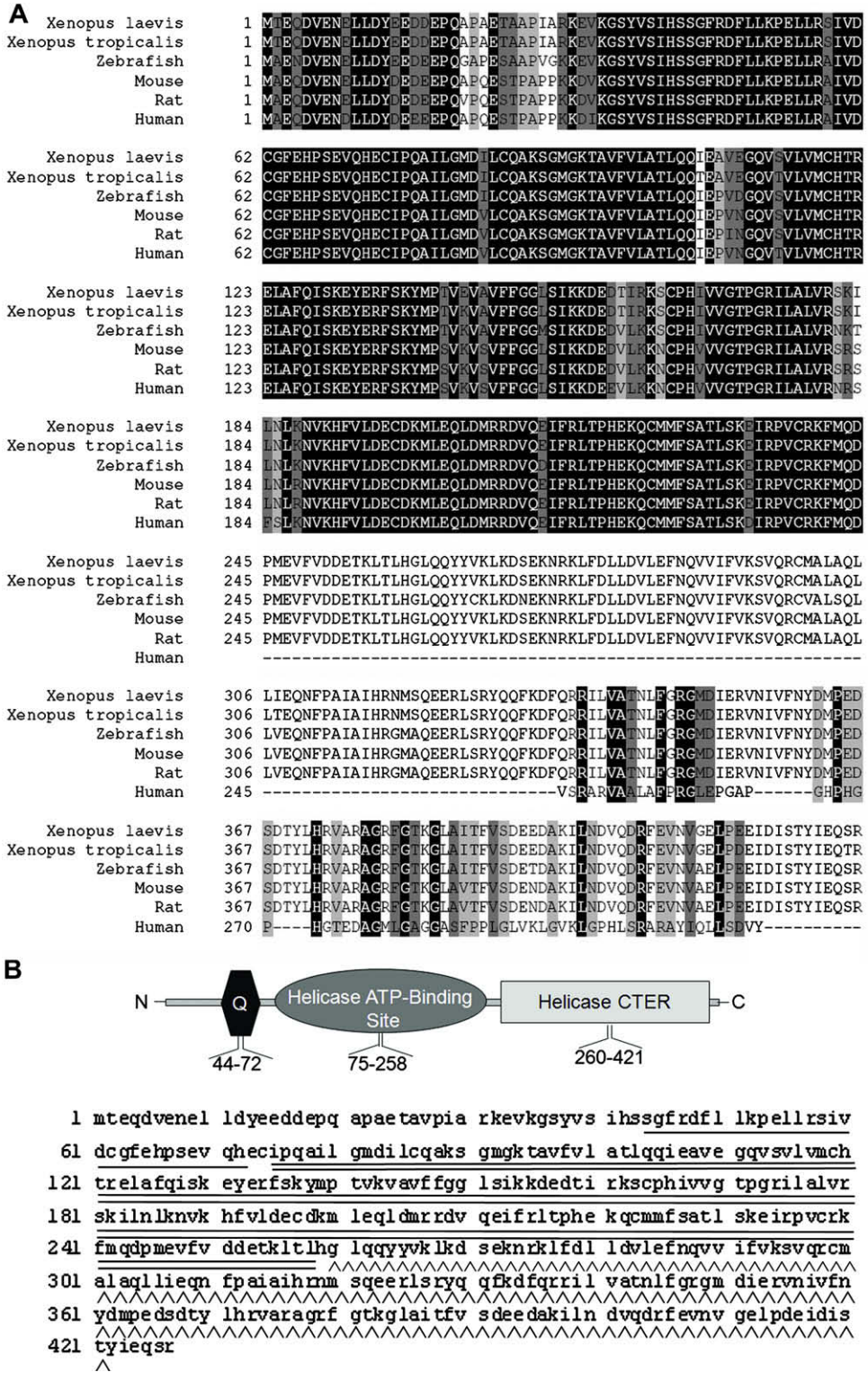

Fig. 1. *Xenopus laevis* DDX39 is a highly conserved DECD Dead-box RNA helicase. (A) The predicted amino acid sequence of *Xenopus laevis* DDX39 aligned with sequences of other vertebrate homologues. Sequence identity, calculated and aligned by Clustal W 1.81 (Higgins et al., 1992; Thompson et al., 1994a,b), with 100% homology of all 6 species are shown in black boxes, while those with strong sequence conservation are shown in dark grey and weak sequence conservation in light grey. Amino acids with little conservation between all 6 species have white background. The number on the left hand side beginning each line indicates the first amino acid positions of that row. (B) Known protein domains in the *Xenopus laevis* DDX39 were identified using the Prosite tool available at the Expasy website (http://ca.expasy.org/tools). The upper part of (B) shows a diagram of identified domains in the *Xenopus laevis* DDX39; Q is a motif peculiar to the Dead-box RNA helicases that is thought to regulate ATP-binding to the downstream helicase ATP-binding site (grey circle; also known as the Walker B motif); at the carboxy terminus is a region homologous to the helicase domain, referred to as the helicase CTER (light grey box). The lower part of B depicts the amino acid sequence of DDX39 with a single underline showing the Q motif, the double-underlined region showing the ATP-binding site and the ^ underlining the region encoding the helicase CTER.