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Cloning of mouse ojoplano, a reticular cytoplasmic protein expressed during embryonic development

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ABSTRACT

Ojoplano (*Opo*) is a morphogenetic gene playing an important role during embryogenesis in medaka. This report focuses on the identification and characterization of the mouse *Opo* gene. We examined *Opo* expression by whole-mount *in situ* hybridization and *in situ* hybridization on sagittal sections during mouse embryogenesis. First expression in whole-mounts was detected at Theiler stages 15–17 (E 9.5–10.5 dpc) as a spotted specific staining in migrating neural crest cells and in placodal structures. A complex expression pattern was observed in Theiler stage 22–23 (E 14.5 dpc) in sagittal sections, including expression in skeletal structures (skull, vertebrae, ribs, bones of the locomotor system), in the nasal region, the heart and the eye. Fusion proteins revealed the localization of OPO within the cytoplasm with a reticular distribution that largely overlapped with the endoplasmic reticulum. *Opo* shows homology to human transcripts linked to a hereditary craniofacial malformation, orofacial cleft 1 (*OFC1*). The expression of mouse *Opo* in neural crest derivatives and skull elements further supports this link.

1. Results and discussion

Ojoplano (*Opo*) was originally identified in medaka as a gene involved in the control of morphogenetic processes during embryogenesis. Due to the unique ocular phenotype observed in medaka *opo* mutants, initial work was aimed to understand the role of the gene in eye development. Besides eye malformation, which inhibits optic cup folding, additional morphogenetic defects were observed in the brain, heart, fins and the craniofacial region of medaka mutants (Martinez-Morales et al., 2009).

At its 5′ end, medaka *opo* displays homology to human *OFCC1*, a candidate gene for orofacial cleft 1 (*OFC1*), OMIM 119530. Translocation breakpoints associated with this disease were identified on human chromosome 6p24 (Davies et al., 2004), which includes the human *OPO* locus. Additional data show that the complex del(6)p syndrome that affects the same genetic locus is associated with abnormal sutures, craniofacial malformations and various eye and ear abnormalities (Palmer et al., 1991). All these phenotypic features resemble those described in medaka *opo* mutants (Martinez-Morales et al., 2009).

Up to now, no mammalian ortholog of medaka *opo* has been studied. To gain insight into the possible functions of *opo* in mammals, we identified and characterized the mouse *Opo* gene and report its expression pattern by *in situ* hybridization. In addition, we generated a GFP-fusion protein to study the subcellular localization of the OPO mouse protein *in vitro*.

1.1. Identification, cloning and bioinformatic analysis of mouse Opo cDNA

The *Opo* cDNA was isolated from E 14.5 mouse embryo mRNA by RT-PCR using specific primers generated against the hypothetical sequence of *Opo* in mouse. The theoretical structure of the gene in mouse was inferred on the basis of available medaka and rat expressed sequence tags (ESTs) and *in silico* predictions using GENSCAN (Burge and Karlin, 1997) and GeneWise (Birney et al., 2004). The cloned cDNA sequence of the mouse *Opo* gene encompassed 2816 bp, including a 2781 bp ORF. Further analysis showed that

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the gene is comprised of 19 exons (chr13:40097251–40383380 assembly mm9). In addition, a short transcript isoform comprised of 14 exons could be identified, containing an ORF of 2361 bp. Both transcripts, designated *Opo* variant A (long isoform) and *Opo* variant B (short isoform) shown in Fig. 1B were deposited in GenBank Accession Nos. EU683438 and EU683439.

The putative OPO isoform A protein encompasses 926 amino acids with a calculated molecular weight of 105 kDa. Isoform B contains 786 amino acid residues with a predicted molecular weight of 90 kDa. By ClustalW alignments, the orthologous proteins of mouse and medaka show 34.6% and 32.7% identity for the isoforms A and B, respectively. The highest similarity between the orthologous sequences can be found in the C-terminal region (Fig. 1A), which contains four putative transmembrane domains predicted by Phobius (Kall et al., 2004). An additional region of higher conservation can be found near the N-terminus. Both regions of high similarity can be found in other vertebrates (e.g. chimpanzee, rat, guinea pig, cow and horse) suggesting the conservation of *Opo* throughout the vertebrate group.

1.2. Expression of Opo mRNA during embryonic development

Elucidation of the *Opo* expression pattern was carried out by non-radioactive whole-mount *in situ* hybridization (ISH) of mouse embryos from Theiler stages (TS) TS12 (E 8.0 dpc), TS13 (E 8.5 dpc), TS15 (E 9.5 dpc), TS15–16 (E 9.5–10.0 dpc) and TS17 (E 10.5 dpc) (Fig. 2) as well as by ISH on sagittal sections of mouse TS22–23 (E 14.5 dpc) embryos (Fig. 3), covering the whole embryo. ISH detection of *Opo* transcripts showed a complex expression pattern during mouse embryogenesis. Due to the high similarity of both *Opo* transcripts, it was not feasible to design isoform specific probes for ISH. A spotted specific staining consistent with expression in neural crest cells was detectable from early TS15 to early TS16. No specific staining could be detected at earlier stages tested (results not shown). In general, the staining was strongest in TS15–16, and included more individual cells than at earlier TS15 stages. The most apparent staining was detected superficially surrounding the otic vesicle. Single stained cells follow a migratory stream towards the dorsal portion of the 2nd, 3rd and 4th branchial arches. The staining was again stronger and detected in more cells in TS15–16 as compared to TS15. At stage TS15–16, signal was detected ventrally in the branchial arches.

Fewer and more scattered single cells were stained superficially in the region of the developing trigeminal ganglion, and in other facial structures, including the olfactory placode. In TS15–16, a few cells were stained superficially over the eye anlagen directly adjacent to the optic vesicle.

Interestingly, a stream of single (probably migrating) stained cells was detected superficially on the right side of the heart overlying the inflow tract/sinus venosus. Furthermore laterally to the aortic vessels in the lower cervical region where the sympathetic chain starts to condense from migrating neural crest cells staining was visible in TS15–16 embryos. Internally, also single cells in the

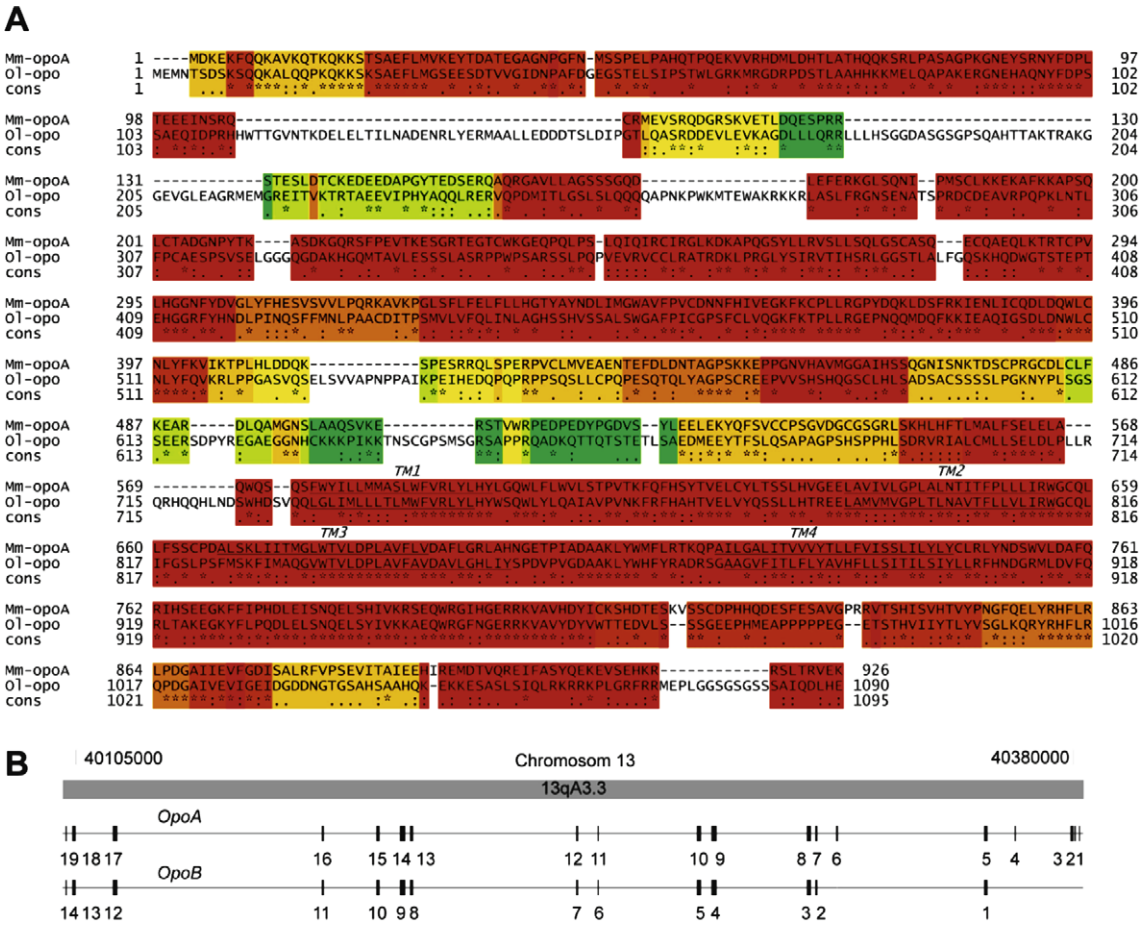

Fig. 1. (A) Comparison of the OPO/Opo protein sequences from mouse (Mm) and medaka (Ol) using T-Coffee (Poirot et al., 2003). Identical amino acids are marked by asterisks, conserved substitutions by dots and semi-conserved substitutions by colons, the quality of the alignment is color coded (BAD–AVG–GOOD) at each position. Transmembrane domains calculated by Phobius for the mouse and medaka proteins are underlined and are labeled TM1, TM2, TM3 and TM4, respectively. (B) Mouse *Opo* locus on mouse chromosome 13, band position qA3.3. The isoforms are distinguished by different 5′ ends and the skipping of an internal exon in *Opo* isoform B.