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Expression of marker genes during early ear development in medaka

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Abstract

Induction of the otic placode involves a number of regulatory interactions. Early studies revealed that the induction of this program is initiated by instructive signals from the mesendoderm as well as from the adjacent hindbrain. Further investigations on the molecular level identified in zebrafish *Fgf3*, *Fgf8*, *Foxi1*, *Pax8*, *Dlx3b* and *Dlx4b* genes as key players during the induction phase. Thereafter an increasing number of genes participates in the regulatory interactions finally resulting in a highly structured sensory organ. Based on data from zebrafish we selected medaka genes with presumptive functions during early ear development for an expression analysis. In addition we isolated *Foxi1* and *Dlx3b* gene fragments from embryonic cDNA. Altogether we screened the spatio-temporal distribution of more than 20 representative marker genes for otic development in medaka embryos, with special emphasis on the early phases. Whereas the spatial distribution of these genes is largely conserved between medaka and zebrafish, our comparative analysis revealed several differences, in particular for the timing of expression.

Keywords: Otic induction; Otic placode; Otic vesicle; Ear, Foxi1; Dlx3b; Medaka; Zebrafish

1. Results and discussion

Ear development is a complex process, controlled by a network of regulatory interactions. In particular the early steps of otic development are highly conserved among vertebrates. During ear formation, presumptive otic cells give rise to an ectodermal thickening called the otic placode, which subsequently develops into the otic vesicle (Barald and Kelley, 2004; Riley and Phillips, 2003; Whitfield et al., 2002). Early transplantation studies in chick embryos revealed that instructive signals are already present before the otic placode forms and are lost during mid-to-late somitogenesis (Groves and Bronner-Fraser, 2000; Kil et al., 2005).

Molecular analysis revealed a number of genes implicated in the induction of this sensory structure. Members of the Fibroblast Growth Factor (Fgf) family of peptide ligands play a key role in this process (Leger and Brand, 2002; Lombardo and Slack, 1998; Maroon et al., 2002; Phillips et al., 2001; Vendrell et al., 2000; Wright and Mansour, 2003). In zebrafish, *Fgf3* and *Fgf8* redundantly induce otic development as could be demonstrated by both gain-of-function as well as loss-of-function experiments (Leger and Brand, 2002; Phillips et al., 2004; Vendrell et al., 2000). Here the signals of the secreted Fgf proteins are mediated within the presumptive otic placodes by the transcription factors *Dlx3b* and *Dlx4b* and independently by *Foxi1* (Liu et al., 2003; Solomon et al., 2004). Another early marker for otic development is *Pax8*, which in zebrafish depends on *Foxi1* function (Solomon et al., 2003, 2004). After this early phase of otic induction, a network of regulatory interactions is initiated at early somitogenesis, which stepwise leads to the formation of substructures within the developing ear. During this process members of the Pax-Six-Eya-Dach regulatory network are thought to play an important role (Riley and Phillips, 2003; Whitfield et al., 2002).

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Much of our knowledge about the genetic hierarchy of otic development originates from studies of a few model organisms. The data known for fish have been obtained from a single model system and in some aspects differ from other vertebrate species. We therefore started to analyse otic induction in medaka fish, a model system distantly related to zebrafish (reviewed in Wittbrodt et al., 2002). The aim of this study was to analyse the expression pattern of otic marker genes during early phases of ear development in a time dependent manner and to compare the results with zebrafish data. In total we analysed more than 20 genes by whole mount *in situ* hybridization from gastrulation until inner ear structures start to form. Expression patterns for the selected marker genes during the induction phase of otic development are presented in Fig. 1. The temporal distribution of their expression from neurula (stage 17) to mid-somi-

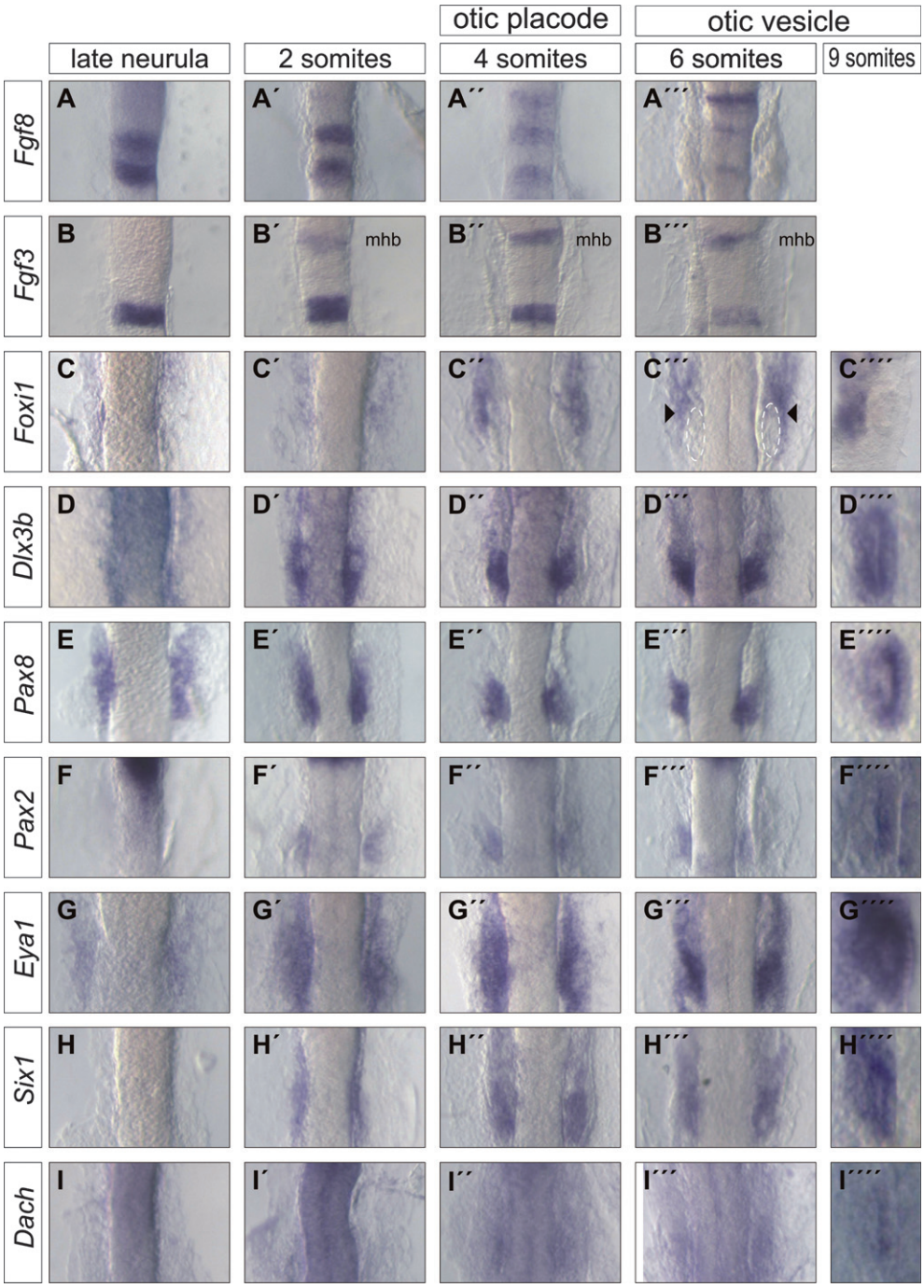

Fig. 1. Marker gene expression during early otic development in medaka. Summary of gene expression patterns involved in otic placode induction. The expression patterns of the indicated genes were analysed by whole mount in situ hybridization from stage 18 (late neurula) until 6 somites. Dorsal views of the otic vesicles (6 somites) at higher magnification are shown for selected genes. The arrowheads in (C‴) indicate the Foxi1 positive territory adjacent to the otic vesicle outlined by a dotted line. Dorsal views for all embryos, anterior to the top; mhb, midbrain–hindbrain boundary; r, rhombomere.