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# Identification and evolution of molecular domains involved in differentiating the cement gland-promoting activity of Otx proteins in Xenopus laevis

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#### ARTICLE INFO

Article history: Received 20 March 2013 Received in revised form 9 September 2013 Accepted 11 September 2013 Available online 20 September 2013

Keywords: Otx Xenopus Cement gland Transcription factors Homeodomain Embryogenesis

#### ABSTRACT

Otx genes are a class of vertebrate homeobox genes, homologous to the orthodenticle gene of Drosophila melanoqaster, that play a crucial role in anterior embryo patterning and sensory organ formation. In the frog, Xenopus laevis, at least three members of this class have been isolated: otx1, otx2 and otx5 (crx); they are involved in regulating both shared and differential processes during frog development. In particular, while otx2 and otx5 are both capable to promote cement gland (CG) formation, otx1 is not. We performed a molecular dissection of Otx5 and Otx1 proteins to characterize the functional parts of the proteins that make them differently able to promote CG formation. We show that a CG promoting domain (CGPD) is localized at the Otx5 C-terminus, and is bipartite: CGPD1 (aa 210-255) is the most effective domain, while CGPD2 (aa 177-209) has a lower activity. A histidine stretch disrupts CGPD1 continuity in Otx1 determining its loss of CG promoting activity; this histidine-rich region acts as an actively CG repressing domain. Another Otx1 specific domain, a serinerich stretch, may also be involved in repressing Otx1 potential to trigger CG formation, though at a much lower level. This is the first evidence that these domains, specific of the Otx1 orthology group, play a role during development in differentiating Otx1 action compared to other Otx family members. We discuss the potential implications of their appearance in light of the evolution of Otx functional activities.

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### 1. Introduction

Otx genes are a class of vertebrate paired-like K<sub>50</sub> homeobox genes (Galliot et al., 1999) related to the *orthodenticle* gene of *Drosophila melanogaster*, which plays a crucial role in the development of the fly nervous system and sensory structures (Cohen and Jurgens, 1990; Finkelstein et al., 1990). Similarly, Otx genes are essential for anterior central nervous system (CNS) and sensory organ formation (Acampora et al., 1995, 1996; Acampora and Simeone, 1999; Freund et al.,

\* Corresponding author. Tel.: +39 0502211497; fax: +39 0502211495. E-mail address: rvignali@biologia.unipi.it (R. Vignali). 1997; Furukawa et al., 1997; Martinez-Morales et al., 2001). In the frog, *Xenopus laevis*, at least three members of the Otx class have been isolated: otx1, otx2, and otx5 (crx) (Blitz and Cho, 1995; Kablar et al., 1996; Kuroda et al., 2000; Pannese et al., 1995; Vignali et al., 2000).

During early phases of *Xenopus* embryogenesis, otx genes are expressed in the developing head tissues (rostral CNS: forebrain and midbrain; anterior endomesoderm of the head organizer) that do not undergo convergent extension movements. Misexpression of these genes by mRNA microinjection

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leads to the development of anteriorized embryos with gastrulation defects (Andreazzoli et al., 1997; Blitz and Cho, 1995; Pannese et al., 1995; Vignali et al., 2000). otx2 prevents cells from performing convergent extension movements through the transcriptional activation of Xenopus calponin homologue cnn1 (formerly XclpH3); calponin impairs movement generation by binding actin and myosin filaments (Morgan et al., 1999). During successive phases of Xenopus development the expression patterns of the three otx genes diverge. In particular, otx2 and otx5 become strongly expressed in the presumptive cement gland (CG) territory, while otx1 is detected in this region only at a much lower level (Blitz and Cho, 1995; Kablar et al., 1996; Pannese et al., 1995; Vignali et al., 2000). Consistent with this, the microinjection of otx2 and otx5, but not of otx1, mRNA leads to the formation of ectopic CGs in the embryo (Andreazzoli et al., 1997; Blitz and Cho 1995; Pannese et al., 1995; Vignali et al., 2000). Therefore, during frog development, these three genes exploit both shared functions (ability to inhibit convergent extension), and differential actions (CG promoting activity). otx2 is able to promote CG formation in naïve ectoderm (Gammill and Sive, 1997, 2001; Sive and Bradley, 1996) where it directly or

indirectly activates the CG markers ag1 (formerly Xag) and muc2 (formerly Xcg), respectively. Sive and coworkers also showed that the 129 C-terminal aa residues of Otx2 are required for this activity, therefore mapping the cement gland promoting region downstream of the WSP domain (Gammill and Sive, 2001). Similar to otx2, otx5 misexpression leads to ectopic CG formation; because of otx5 strong expression in the cement gland anlage, this suggests that otx5 plays a similar role to that of otx2, while otx1 is not able to activate CG formation. However, otx2 and otx5 show also divergent functions. In fact, their misexpression in the frog retina has different effects, with otx2 promoting bipolar cell fate and otx5 promoting photoreceptor cell fate (Viczian et al., 2003). In the retinal context, the different cell fate specification abilities of the two proteins are due to a small divergent region just C-terminal to the homeodomain, that works as a retinal specificity box (RS box) (Fig. 1); when the sequence of the Otx5 RS box is changed into that of Otx2, mutant Otx5 switches its retinal activity to that of Otx2, and vice versa (Onorati et al., 2007). These results altogether suggest that different molecular domains are responsible for the different activities of Otx proteins in the diverse cellular contexts.

	HOMEODOMAIN	
Otx2	MMSYLKQPPYAVNGLSLTASGMDLLHQSVGYPATPRKQRRERTTFTRAQLDILEALFAKT	60
Otx5	MMSYIKQPHYAVNGLTLAGTGMDLLHSAVGYPTNPRKQRRERTTFTRAQLDILESLFAKT	60
Otx1	MMSYLKQPPYGMNGLGLTGPAMDLLHPSVGYPATPRKQRRERTTFTRSQLDVLESLFAKT	60
	****:*** *.:*** *:***** :****	
	HOMEODOMAIN RS box	
Otx2	RYPDIFMREEVALKINLPESRVQVWFKNRRAKCRQQQQQQ <mark>QQNGGQNKVR</mark> PSKKKTS	
Otx5	RYPDIFMREEVALKINLPESRVQVWFKNRRAKCRQQQQQ	
Otx1	RYPDIFMREEVALKINLPESRVQVWFKNRRAKCRQQQ QQQQQQQQSSSGAGVKSRPAKKKCS   ************************************	120
0+2	PVREVSSESGTSGQFSPPSS-TSVPVISSSTAPVSIWSP	1 5 4
Otx2 Otx5	PARETNSEASTNGOYSPPPPGTAVTPSSSASATVSIWSP	
OLAD	Serine-rich region	100
Otx1		179
	*.** * : :.**: ::** *** ***************	
Otx2	ASVSPLSDPLSTSSS-CMQRSYPMTYTQASGYSQGYAG-STSYFGGM	199
0.5		201
Otx5 Otx1	ASISPIPDPLSAVTNPCMQRSTG <mark>YPMTYSQAPAYTQSYGG-SSSYFTGL</mark> ASISPGTAPGSGPDPLGTGSASCMQRSGSSSAASYPMSYSQAAGYTQAYPAPSSSYFSGV	201 239
ULXI	**:** .***: ***** ***:**.*:**.**	233
Otx2	DCGSYLSPMHHQLSGPGATLSPMGTNAVTSHLNQSPVALSSQAYGASSLG	2/9
ULAZ	CGPD1	
Otx5	DCGSYLSEMHPOLSAPGATLSPIATPTMGSHLSQSPASLSAQGYGAASLG	251
Ot.x1	Histidine-rich region DCSSYLGPMHSHHHPHOLSPMAPSSMSGHHHHHHHLSOTSSHHHHHHHHOGYTSSALP	297
000012	**.*** : ***: :: **.*:. *.*	201
	Otx tail	
Otx2	FNSTDCLDYKDQTASWKLNFNA-DCLDYKDQTSSWKFQVL 288	
_		
Oxt5	FTSVDCLDYKDQTASWKLNFNATDCLDYKDQ-SSWKFQVL 290	
Otx1	FNSS <u>DCLDYKEQATASSWKLNFNSTDCLDYKDQ-ASWRFQ</u> VL338 *_* ******:*: :******: ******* :*******	

Fig. 1 – Multi-alignment of *Xenopus* Otx1, Otx2 and Otx5. Multi-alignment of Otx sequences has been obtained using ClustaW. "": identical residue; ":": conserved substitution; ".": semi-conserved substitution. Homeodomain: shaded dark grey box; Otx tail: shaded light grey box; Otx2 and Otx5 retinal specificity boxes (RS box): shaded yellow boxes; Otx1 Ser-rich region: open box; Otx1 His-rich region: light green box; cement gland promoting domain D2 (CGPD2): shaded light blue box; cement gland promoting domain D1 (CGPD1): shaded blue box.

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