

## CRUCIAL ROLES OF RETINOIC ACID SIGNALLING DURING TEETH INDUCTION IN TELEOST FISH

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**INTRODUCTION:** In contrast with other teleosts, the zebrafish has teeth only localized in the pharynx. FGF signalling has been shown to be crucial during pharyngeal and oral teeth induction in teleosts [1,2]. In absence of this signal, several genes including *dlx2a* and *dlx2b* required for teeth development are no longer expressed and teeth are absent. To date, no other signalling pathways have been investigated during fish teeth induction. Since retinoic acid (RA) has pleiotropic functions during vertebrate development we investigate its precise roles during teleosts teeth induction. We used several fish models with teeth only in the pharynx or teeth located both in the pharyngeal and oral cavity. RA is synthesized by *aldh1a* enzymes (four in mammals) and binds RA receptors that are ligand-dependant transcription factors.

**RESULTS & DISCUSSION:** We used the zebrafish *neckless* (*nls*) mutant, which is devoid of full retinoic acid production from the RA-synthesizing enzyme *aldh1a2* to show that teeth induction does not occur in absence of RA signalling.

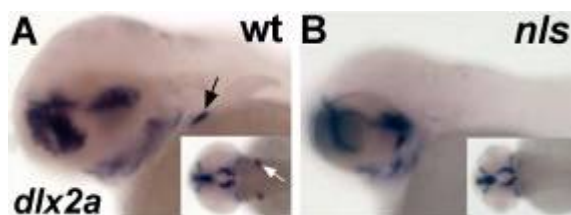


Fig. 1: Teeth induction does not occur in *nls* mutant. (A) wild type embryo with *dlx2a* expression in the first pair of teeth (arrows). (B) *nls* mutant with lack of *dlx2a* expression in the teeth.

By abolishing RA signal using pharmacological treatments at different times during embryonic development, we showed that RA is necessary during three different phases for the induction of the first pair of teeth in zebrafish: 1- for the proper migration of the neural crest cells (NCCs) at around 10 hpf, 2- for the maintenance of the NCCs in the ventral

posterior pharynx at 26 hpf and 3- for the first pair of teeth induction *per se* at 43 hpf.

We further demonstrate that the third phase of RA (the teeth induction phase) is solely dependant on *aldh1a2* and not of any other *aldh1a* enzymes. By abolishing other cellular domains of *aldh1a2* expression, we show that the origin of RA signalling is located in the ventral posterior pharynx close to the fifth ceratobranchial arch.

We next demonstrate that RA and FGF signalling are not required at the same developmental time for the induction of the first pair of teeth and that FGF signalling acts downstream of RA signaling during teeth induction.

Finally, we used two other teleost fish, the medaka *Oryzias latipes* and the mexican tetra *Astyanax mexicanus* that possess teeth in both the oral and pharyngeal cavity to determine the roles played by RA in oral teeth induction. We showed that the NCCs phases only affect the induction of the pharyngeal teeth and not the oral teeth induction. Furthermore, we demonstrate that oral teeth induction is RA independent.

**CONCLUSIONS:** We uncover new roles for RA signalling in the induction of the first pair of teeth. We show that RA signaling is generated via the RA-producing enzyme *aldh1a2* in the ventral posterior pharynx. In an evolutionary perspective, we show that similar tissue like teeth are dependant on different signaling pathway for their induction depending in their localization.

**REFERENCES:** <sup>1</sup> W.R. Jackman, B.W. Draper, and D.W. Stock (2004) *Dev. Biol.* **274**:139-157. <sup>2</sup> D.W. Stock, W.R. Jackman, and D. Trapani (2006) *Development* **133**:3127-37. <sup>3</sup>

**ACKNOWLEDGEMENTS:** We thank Jean-Stéphane Joly and Sylvie Retaux for help with medaka and mexican tetra, respectively. This work was supported by the Ecole Normale Supérieure de Lyon, the Region Rhone-Alpes, the "Agence Nationale pour la recherche" and an ARC grant to YG.