Comparative genomics reveals miRNAs that give identity to developing mid-facial tissues

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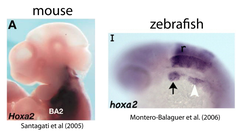
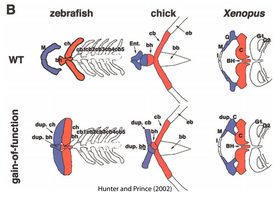
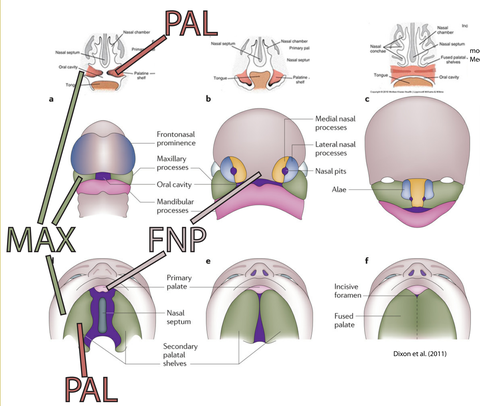
1 Do different regions of the developing craniofacial complex get their identity through the action of microRNAs (miRNAs)?

a Different regions of craniofacial complex have genetic "identity"



Santagati et al (2005) Montero-Balaguer et al. (2006)

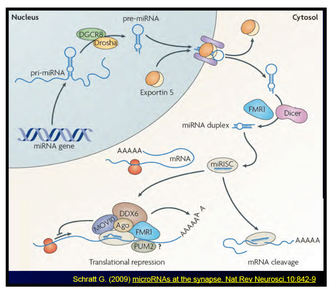




b Identity of mid-facial tissues is crucial for development of the palate

FNP=frontonasal process
MAX=maxillae
PAL=palatal shelves

c miRNAs regulate gene activity

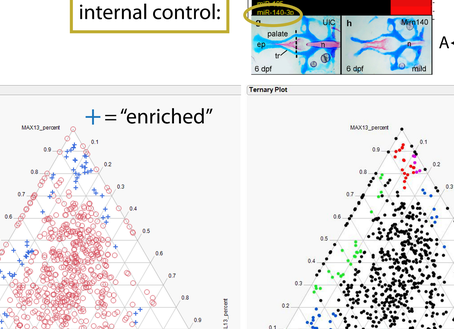


a + **b** + **c**

Hypothesis: miRNA sub-populations drive palatal development by giving identity to mid-facial tissues

2 Identifying mid-facial tissue miRNAs by miRNAseq

dissect tissues & purify small RNAs



	total # sequences*	# sequences removed bioinformatically**	# remaining that match to miRBase	unique miRBase genes matched	# remaining that don't match to miRBase
E10.5 FNP	24,848,524	19,000,820	3,635,484	**640**	2,212,220
E10.5 MAX	19.889,352	13,426,350	3,412,840	**605**	3,050,162
E11.5 FNP	20,842,495	17,519,863	1,983,290	**598**	1,339,342
E11.5 MAX	24,842,312	20,135,538	2,544,990	**636**	2,161,784
E12.5 FNP	34,502,816	19,839,537	11,094,564	**885**	3,568,715
E12.5 MAX	49,239,804	28,040,116	15,935,421	**834**	5,264,267
E12.5 PAL	60,264,978	42,449,718	13,051,637	**852**	4,763,623
E13.5 FNP	32,749,316	14,594,970	14,194,885	**836**	3,959,461
E13.5 MAX	33,237,037	20,169,602	10,239,997	**809**	2,827,438
E13.5 PAL	27,601,010	10,859,190	13,386,617	**771**	3,364,203
E14.5 MAX	65,654,863	25,402,008	33,316,722	**938**	6,936,133
E14.5 PAL	38,856,026	15,083,846	19,196,331	**831**	4,575,849

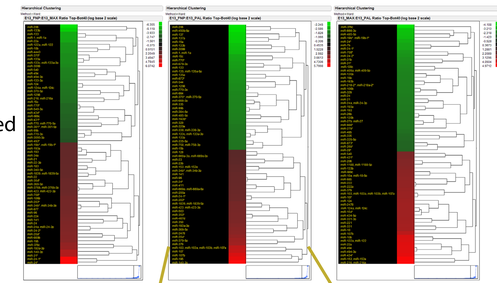
*after removal of sequences with N's or without barcodes

**i.e., those sequences without adapters, not 18-24 long, or counts <30

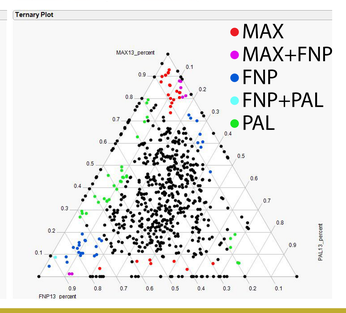
3 Bioinformatics reveal tissue-specific enrichment of mid-facial miRNAs

E13.5 sample comparison: FNP:MAX FNP:PAL MAX:PAL

green: under-expressed
red: over-expressed



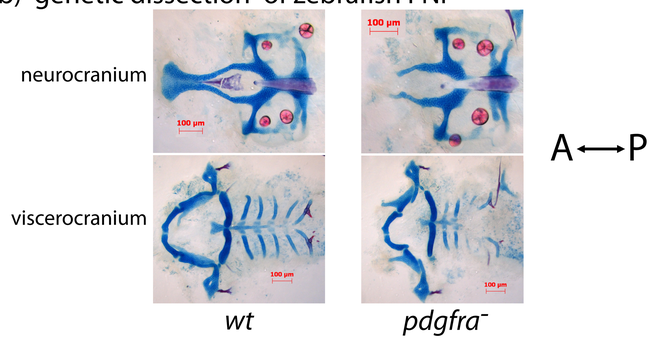
internal control:



4 Future directions

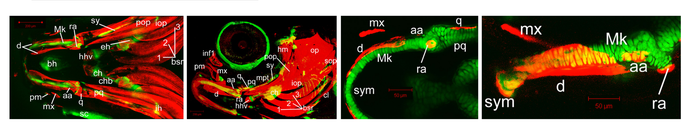
a) refine bioinformatic pipeline (see Batzel et al. poster)

b) "genetic dissection" of zebrafish FNP



c) Testing function of enriched miRNAs by injecting miRNA duplexes in zebrafish (see Ding et al. poster)

5 FishFace Atlas



https://www.facebase.org/fishface/home

Also interested in teleost craniofacial morphology? Check out another FaceBase project (from Kimmel lab)

Abstract

Defects in mid-face development, including cleft lip/palate, account for the largest number of birth defects annually. Understanding the molecular genetic mechanisms that lead to human clefting remains a challenge because the development of the palate involves many interacting genes and craniofacial disease may involve slight modulation of these genes rather than total abrogation. MicroRNAs (miRNAs) are short, non-protein-encoding RNAs that modulate gene expression post-transcriptionally in numerous developmental and physiological processes.

To explore the hypothesis that the mis-regulation of miRNAs underlies cleft palate, we sought to identify and analyze miRNAs that define different regions of the mid-face. We dissected palatal shelves (PAL), maxillae (MAX), and frontonasal prominences (FNP) from E13.5 mouse embryos, isolated their populations of miRNAs, and subjected samples to deep sequencing. We developed a bioinformatics pipeline (see abstract by Batzel et al.) that identifies miRNAs, quantifies gene expression, and compares results among these three regions of the mid-face. The pipeline identified groups of miRNAs that are down-regulated or up-regulated specifically in each of the three individual craniofacial tissues relative to the other two. Our pipeline also compared sequenced libraries to the mouse genome and thereby revealed many potential unannotated mouse miRNAs expressed in developing craniofacial tissues. Finally, we analyzed the frequency and type of RNA editing employed in each miRNA population, looking for miRNA-mediated editing mechanisms that may be specific to a given mid-facial region. These results offer the first detailed look at the microRNA content of specific midfacial organs, and importantly, provide a list of miRNAs for functional tests in zebrafish for their roles in regulating normal palatogenesis.