• RNA dynamics in the developing mouse face

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Aim 1. Describe the transcriptional dynamics of mouse facial development.

What are the genes and gene regulatory networks that enable the various prominences to give rise to different derivatives?

Aim 2. Experimental and bioinformatics analysis of differential splicing.

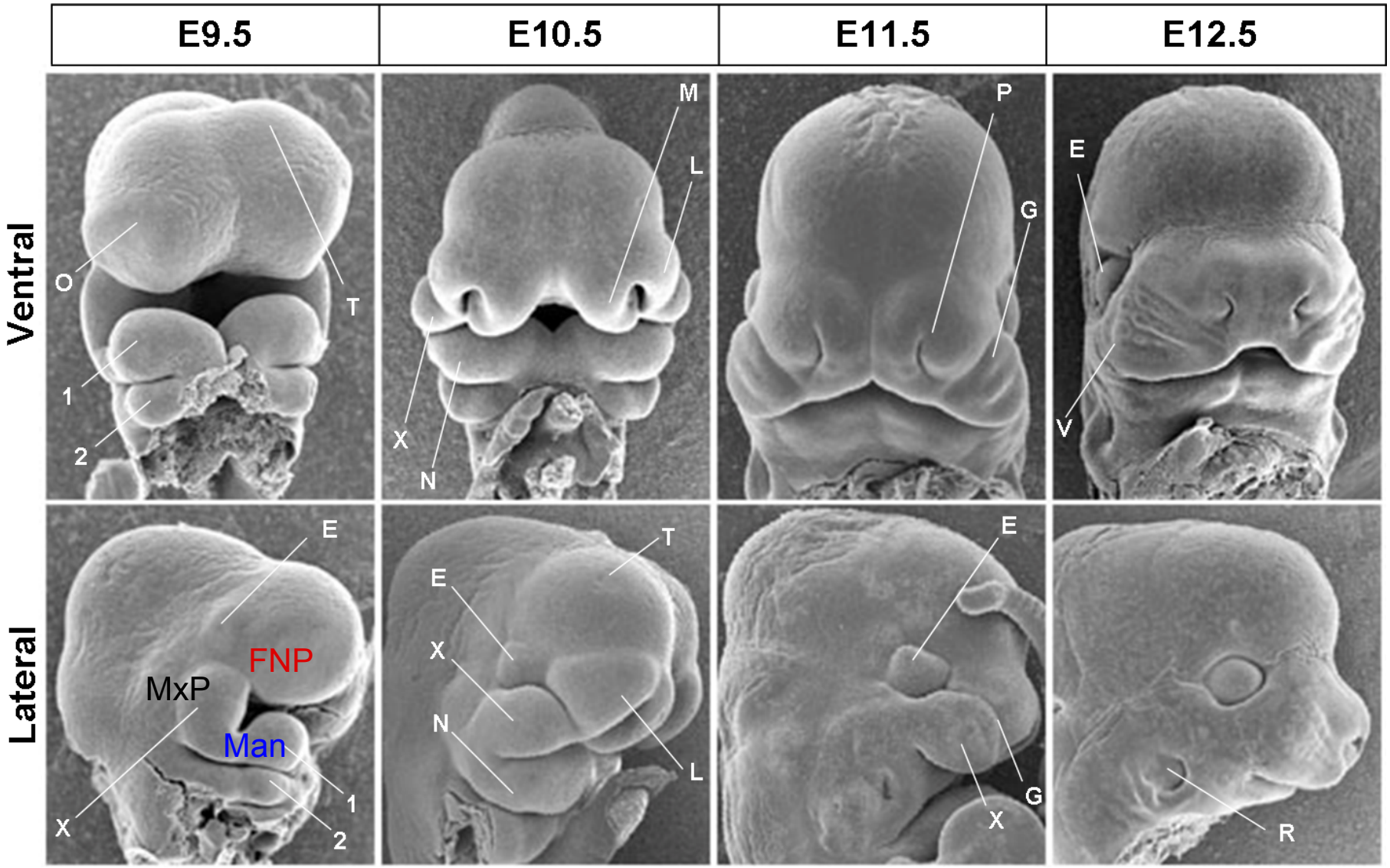
Loss of epithelial-specific splicing factors causes drastic changes in face formation (R. Carstens) – what isoform differences distinguish the various tissues and prominences over time?

Aim 3. Describe the post-transcriptional RNA dynamics of mouse facial development.

Not today!

Normal Craniofacial Development

(mouse)



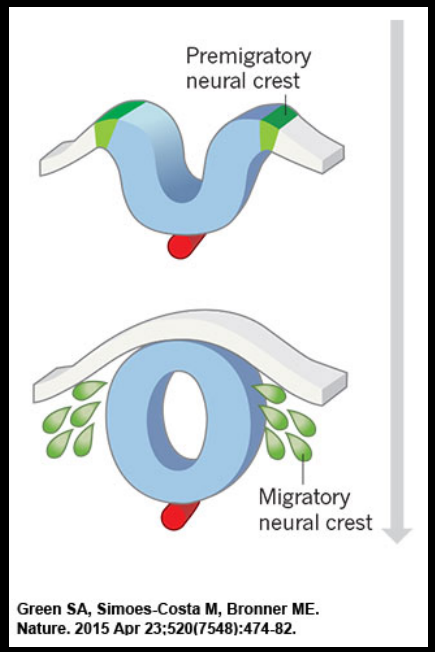
FNP = Frontonasal

MxP = Maxillary

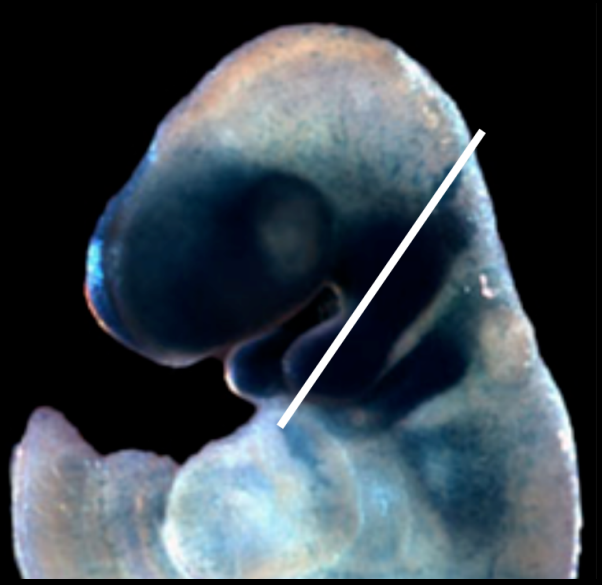
Man = Mandibular

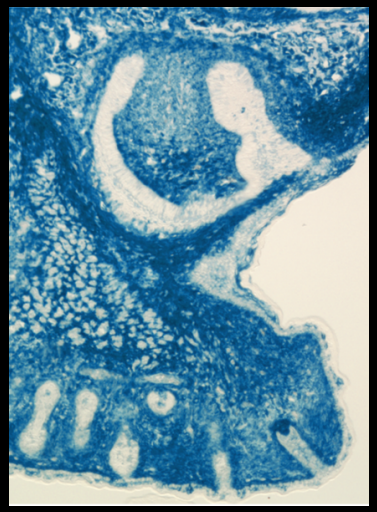
Matt Kaufman

Tissue Interactions During Face Formation



Green SA, Simoes-Costa M, Bronner ME.
Nature. 2015 Apr 23;520(7548):474-82.



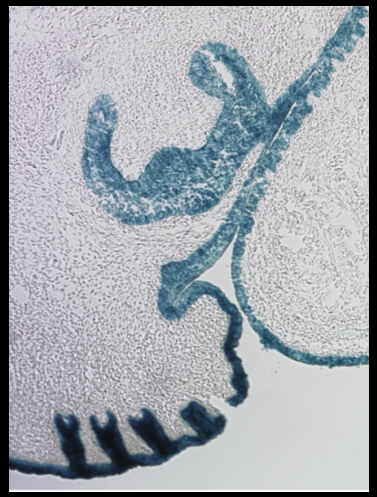


Neural crest

Neural Crest and Mesoderm form the Mesenchymal cell populations that are the building blocks of the face – they will form e.g. skeleton and muscles.

Surrounding tissues, especially ectoderm, provide critical signals for growth, patterning and morphogenesis, as well as contributing to teeth, glands, eyes and hair.

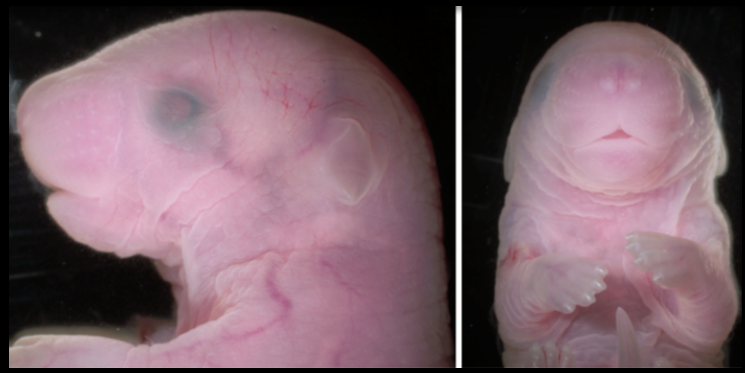
Note: much less ectoderm than mesenchyme is present!



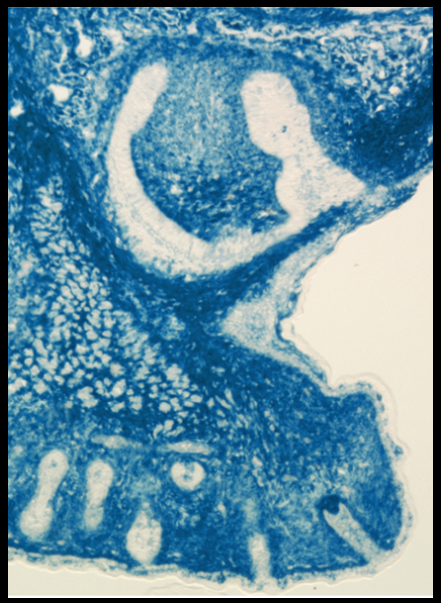
Ectoderm

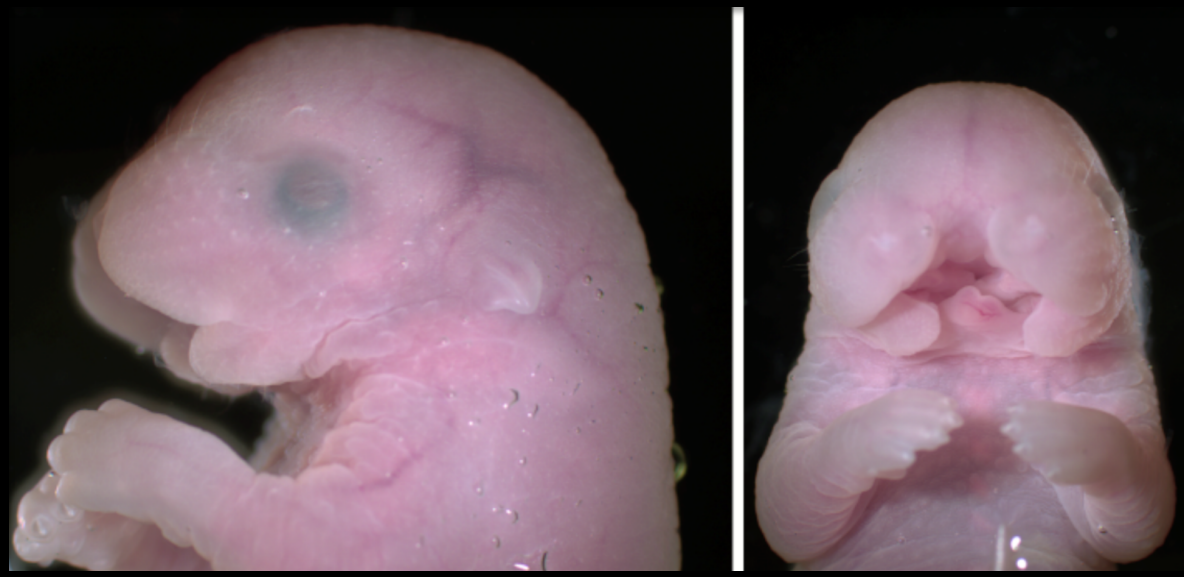
Facial defects arising when AP-2 transcription factors are deleted in the neural crest

CONTROL



Neural-crest KO

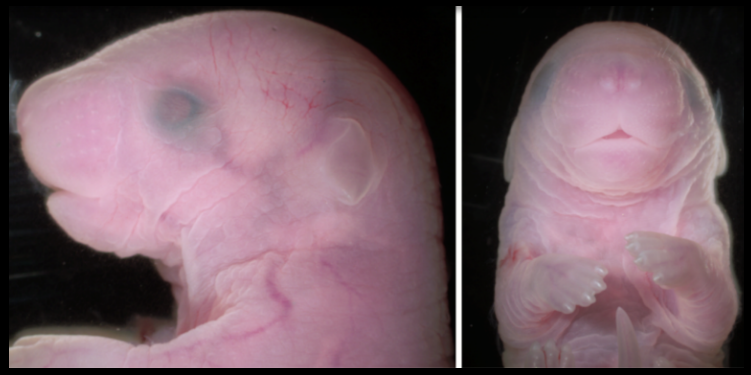




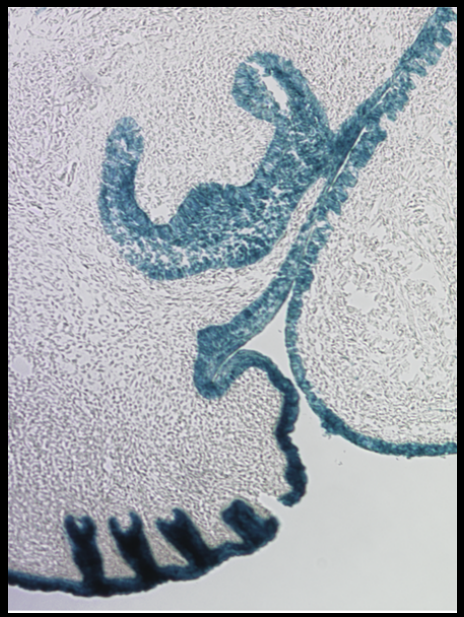
Eric Van Otterloo

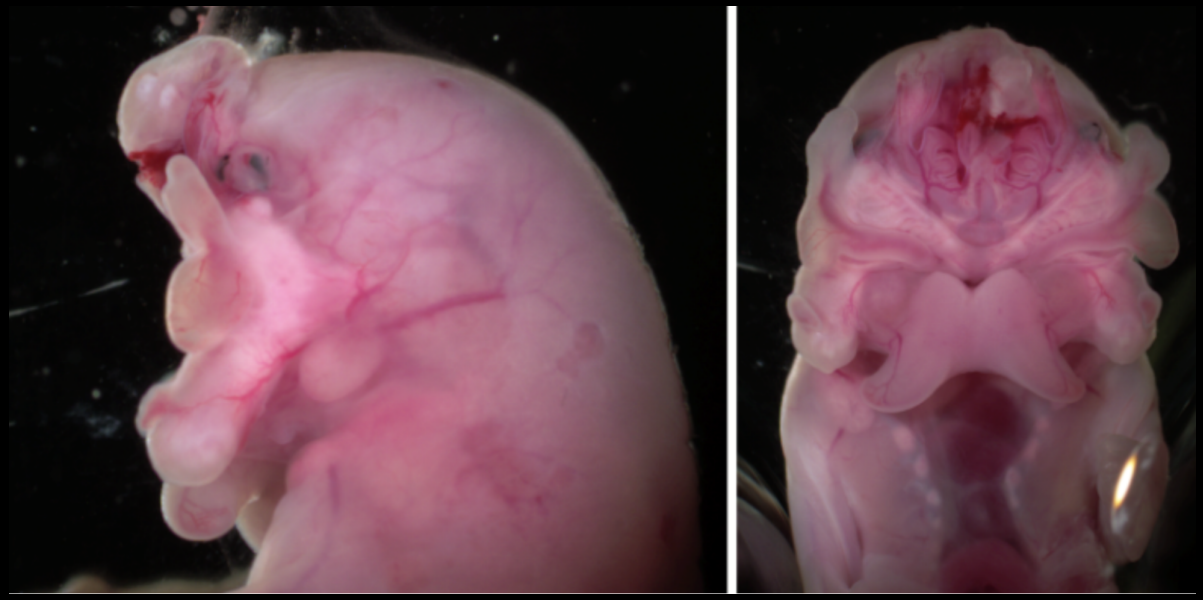
Facial defects arising when AP-2 transcription factors are deleted in the ectoderm

CONTROL



Ectoderm KO





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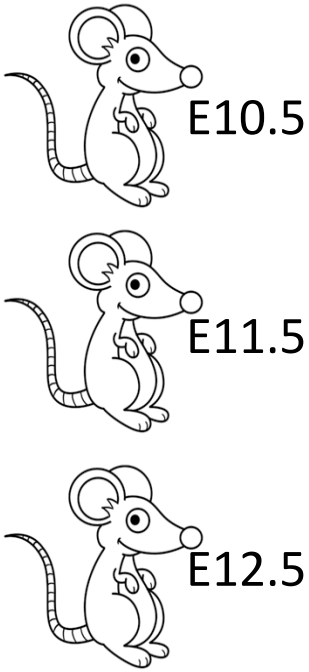
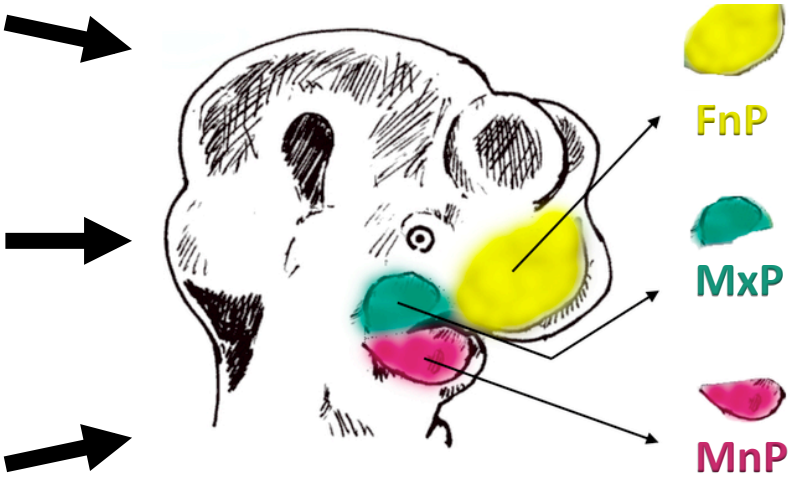
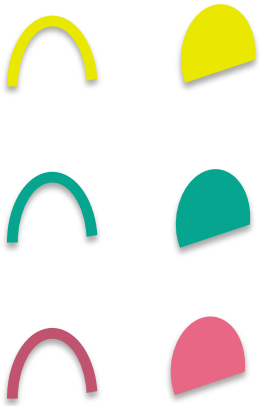
APPROACH

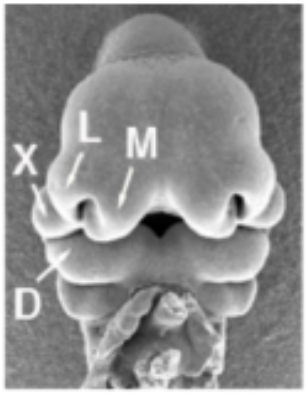


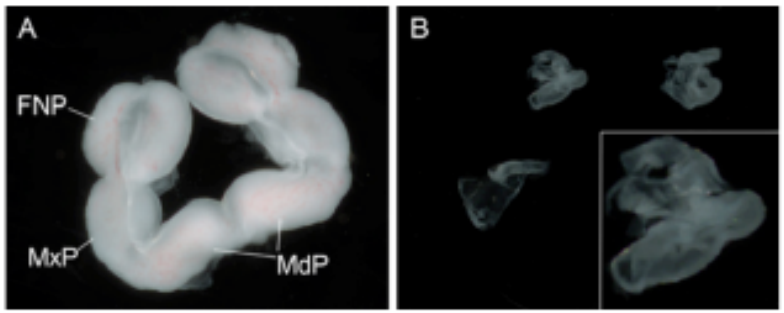


dispase

Ect + Mes



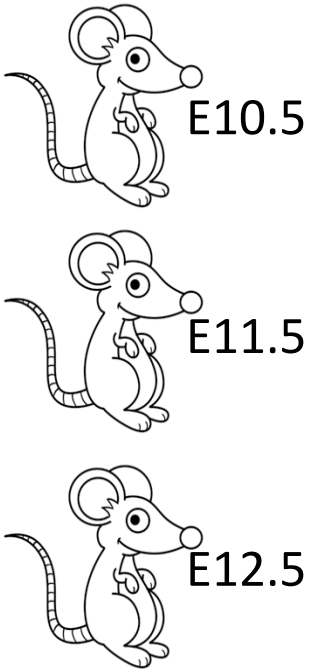


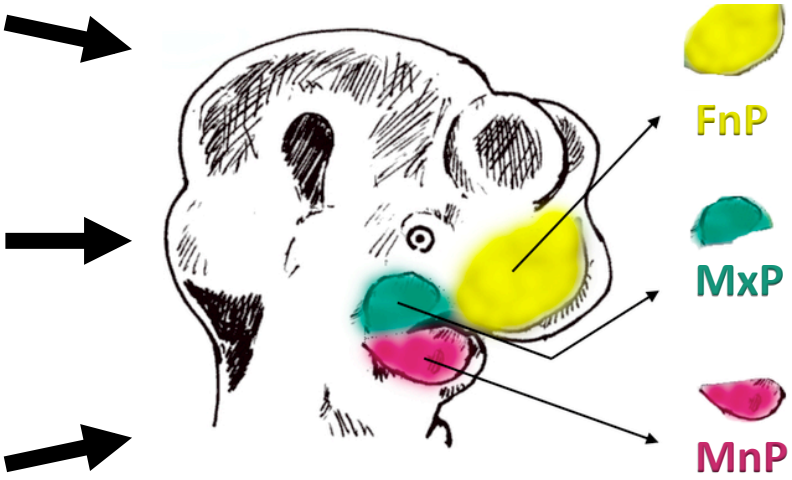


RNA

e.g. mRNA
For RNAseq

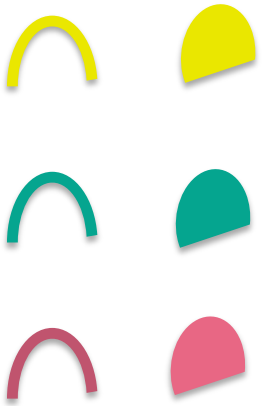
APPROACH

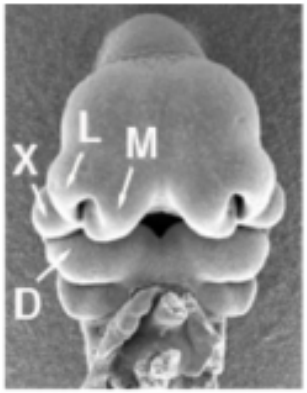




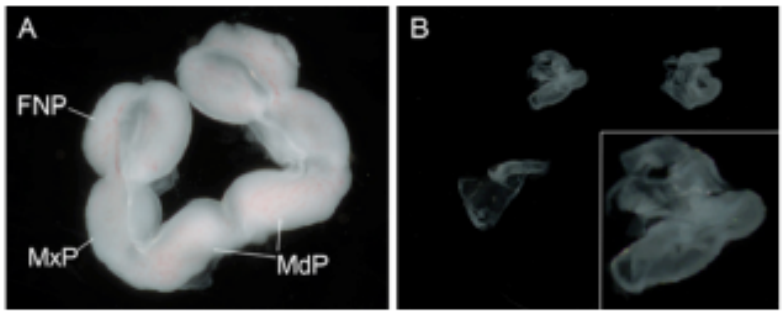
dispase

Ect + Mes





E10.5



Intact Ectoderm

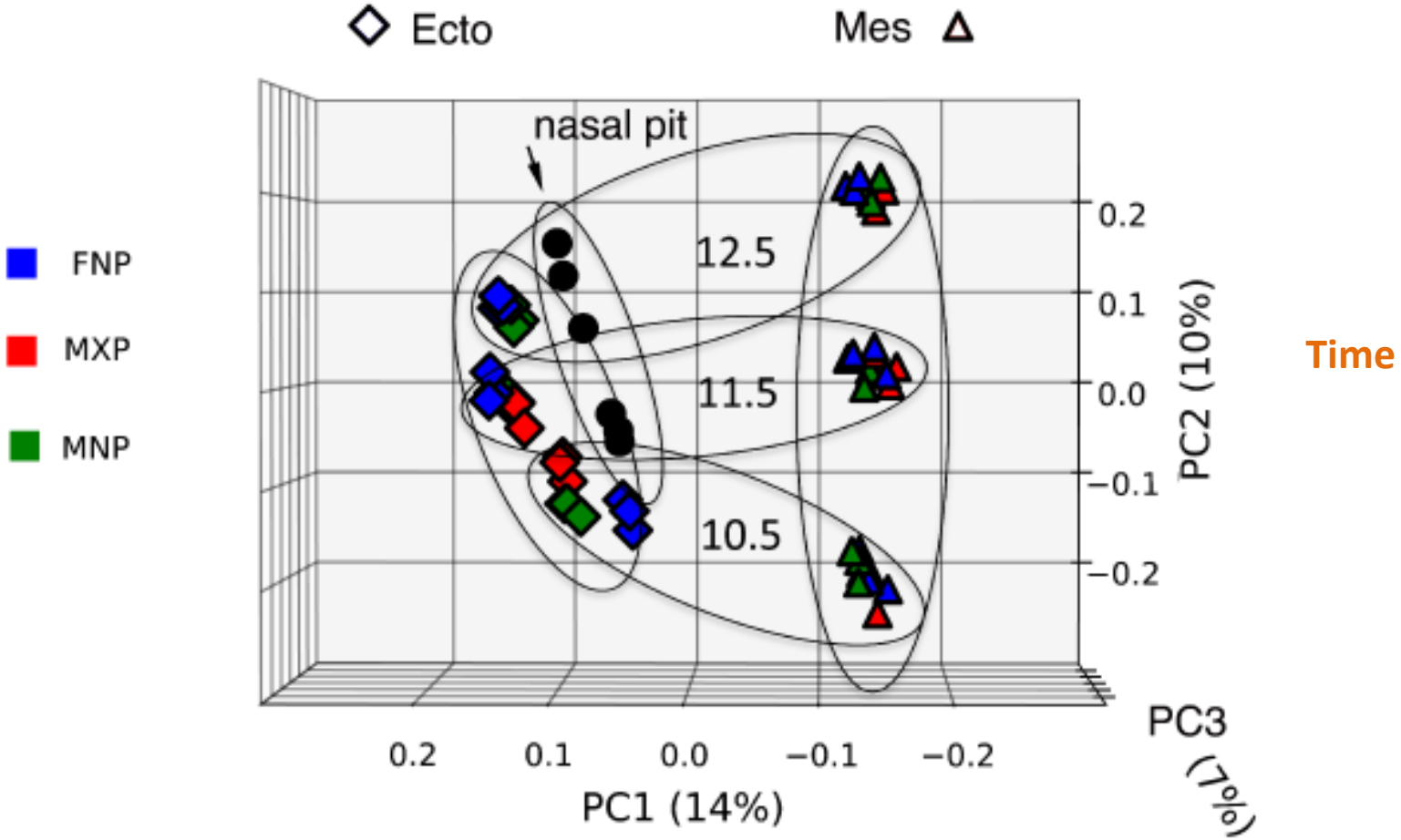
RNA

e.g. mRNA
For RNAseq

COMPLETED!

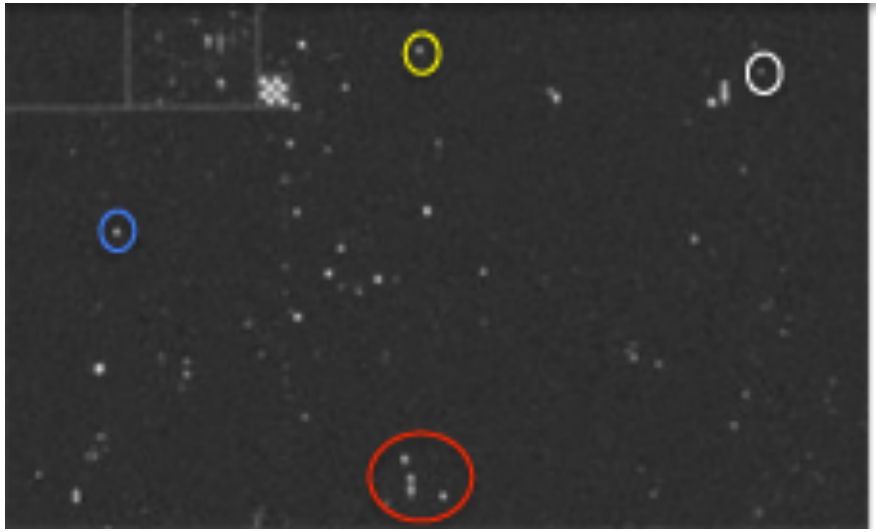
Aim 1. Mouse RNA-Seq Datasets are complete.

Principal component analysis indicates replicates are tight.
Samples separate mainly by tissue layer and time, and to a lesser extent prominence.
Data are suitable for in depth analysis (Aim 2, Joan Hooper)

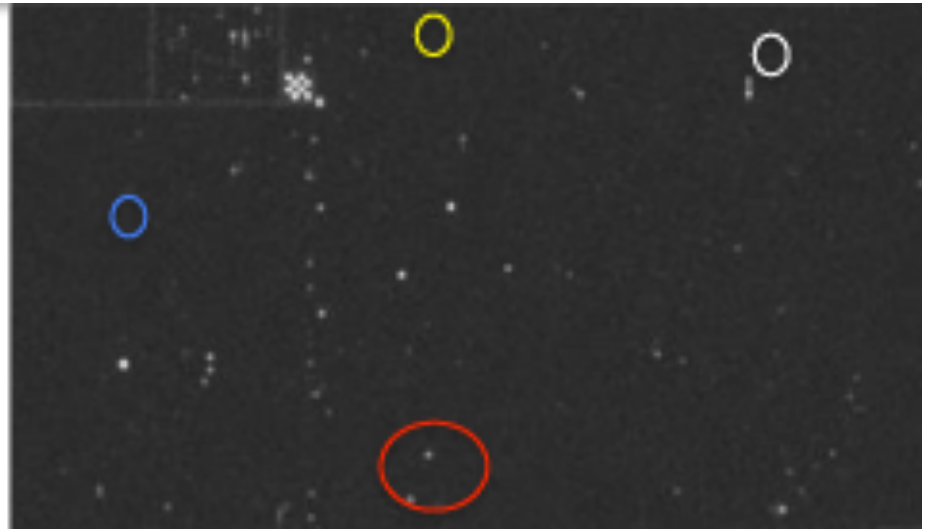


Aim 1. miRNAs and other small RNAs

- Critical regulators of face formation, but not poly-adenylated.
- Analyze by sequencing or microarray?
- We decided on microarray for a combination of technical reasons, including the ability to detect a broader range of RNA classes, as well as reproducibility.



Dicer plus



Dicer minus

miRNAs and other small RNAs

In association with Affymetrix, we designed a custom mouse small RNA array

Category	Sequences	Probes per probe set	Probe count
miRNA mature from miRBase release 21	1,915	9	17,235
miRNA hairpins from miRNA 4.0	1,174	11	13,768
miRNA hairpins new in miRBase release 21	8	11	88
miRNA hairpins recovery of poor sequences	11*	Varies	89
tRNAs from gtRNAdb	471	11	5,457
Additional Mt tRNAs/controls from NCBI	4	11	44
MTA TCs for small RNAs**	3,383	Varies	125,077
MTA junctions for small RNAs**	2,670	4	10,657
Enhancer RNAs forward direction	3,943	Every 100 bases	77,805
Enhancer RNAs reverse direction	3,943	Every 100 bases	77,845
Total			**328,065**

Total capacity of 100/9 format array at 6-micron features is 365k probes

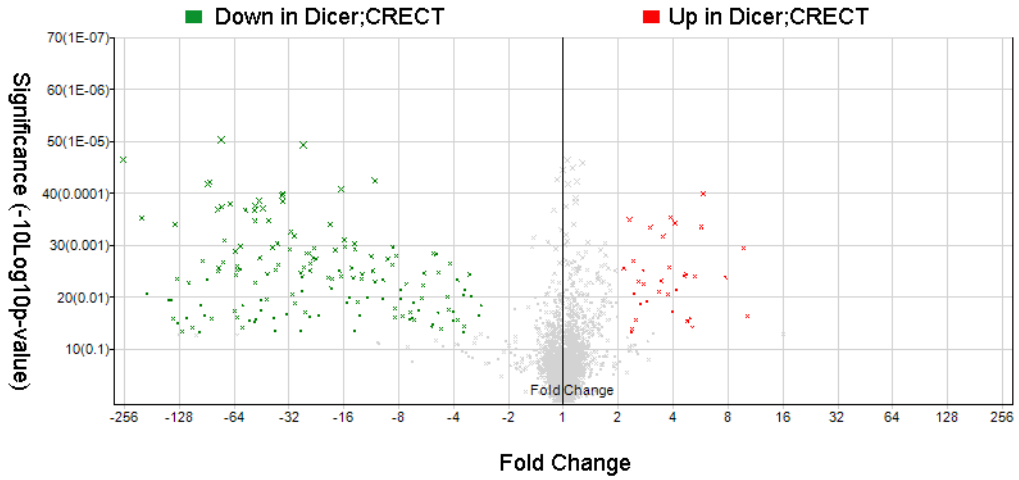
*12 sequences total, 1 sequence had no valid probes

**Non-Mt tRNA content removed from MTA TCs and junctions

We also worked with Dr. Visel's group to add eRNAs to the array for a pilot studies on whether these enhancer associated transcripts can be detected and characterized using this technological approach.

Preliminary data: miRNAs and other small RNAs

- We used biological triplicate samples from E16.5 secondary palate ectoderm for a test run.
- We can detect strong signal from: miRNAs, tRNAs, snoRNAs, snRNAs, 5S RNA.
- Data from biological triplicates are reproducible.
- Preliminary analysis indicates we can detect many miRNAs known to be expressed in skin and palate, e.g. mir-203, mir-200c, mir-205.
- Analysis of equivalent tissue from Dicer KO embryos show significant and specific loss of miRNA signals.



PLANS: Troubleshoot eRNA signal.

Utilize arrays for the samples:
TIME
TISSUE
PROMINENCE

Aim 2: Differential Isoform Analysis…

Initial analysis of isoforms with differential expression.

These examples represent differences for ectoderm vs mesenchyme shown using IGV browser screenshots.

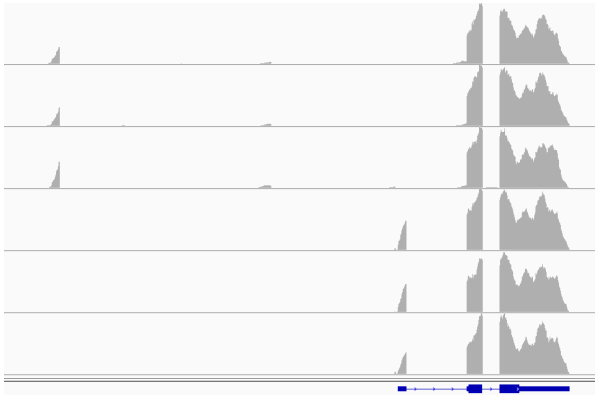
These represent functionally significant examples.

Reproducible data-ready for in depth bioinformatic analysis.

Ecto x 3

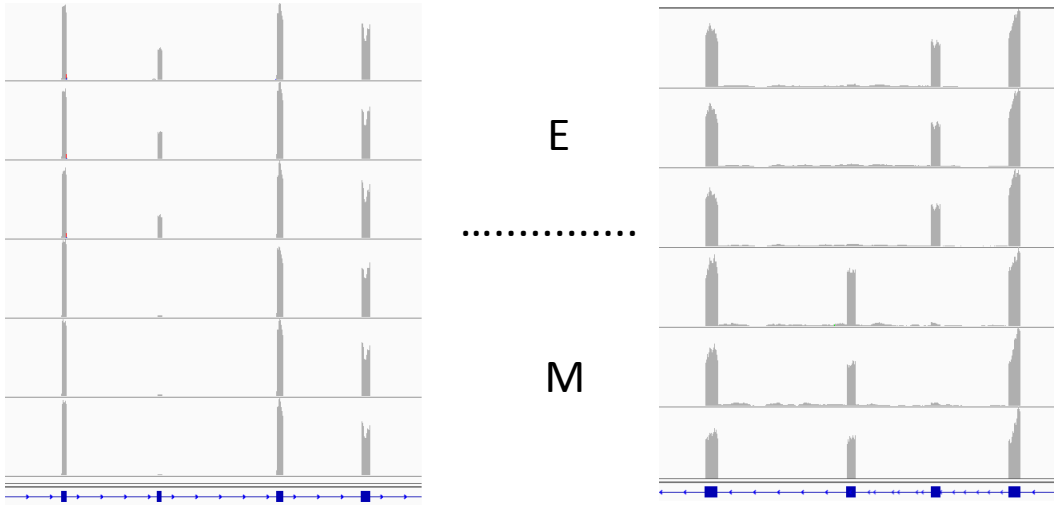
……

Mes x3



Differential promoter usage

S100a16



E

……

M

Differential splicing

Lef1

Fgfr2

Preliminary Differential Isoform Conclusions

- Most differential isoform usage occurs between the different tissue layers.
- Many of the corresponding genes are associated with EMT in other biological contexts.

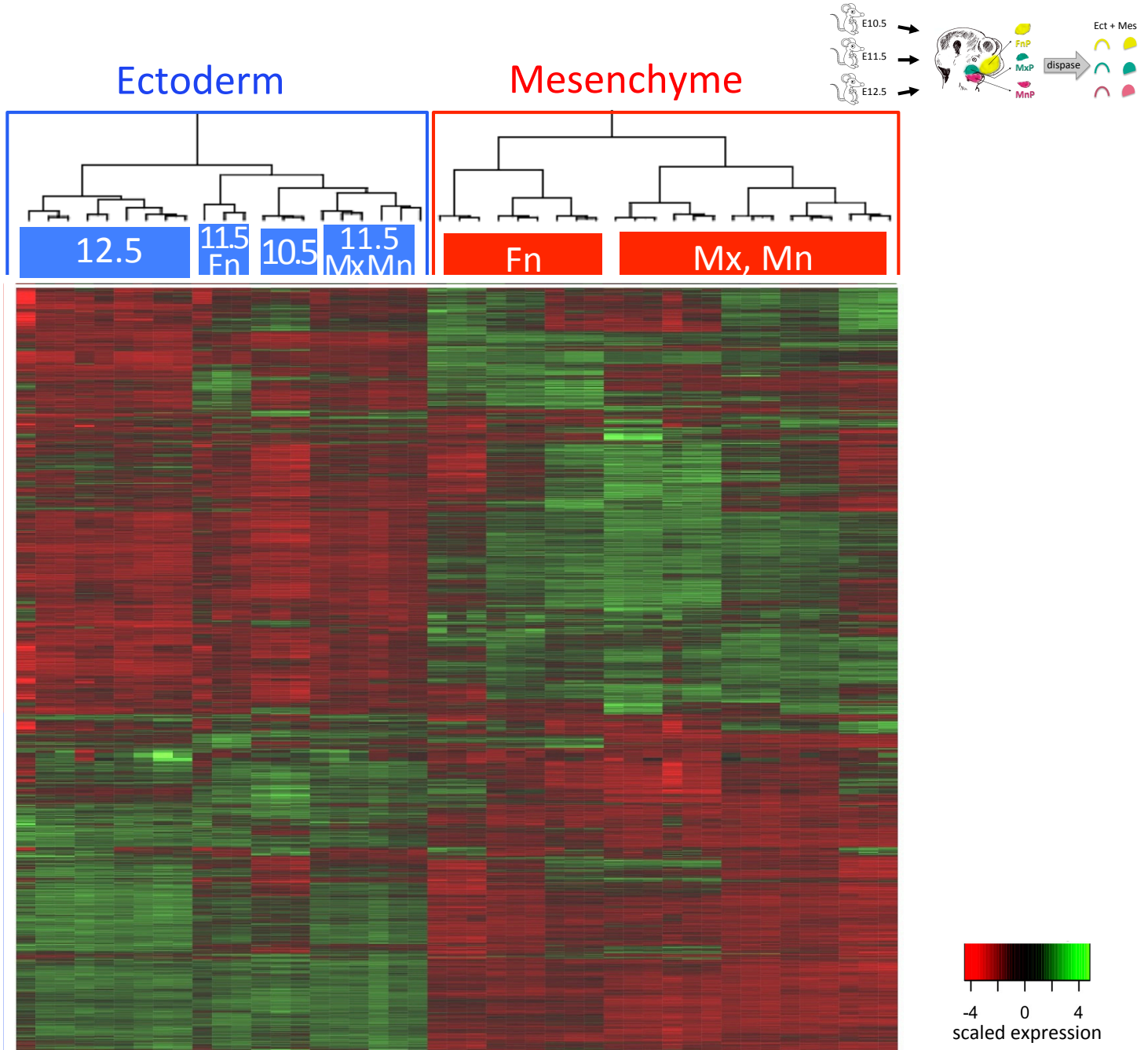
- There are few isoform differences between the common transcripts expressed in the three facial prominences.

- A few genes show alterations in exon usage over time in a specific tissue.
- Such “time” genes often show “sloppy” splicing of particular exons over time with more retained intron sequences.

- PLANS: Additional bioinformatic and experimental analysis planned to characterize and validate these isoform differences using transcriptome arrays, RT-PCR etc.

beyond the gene lists…

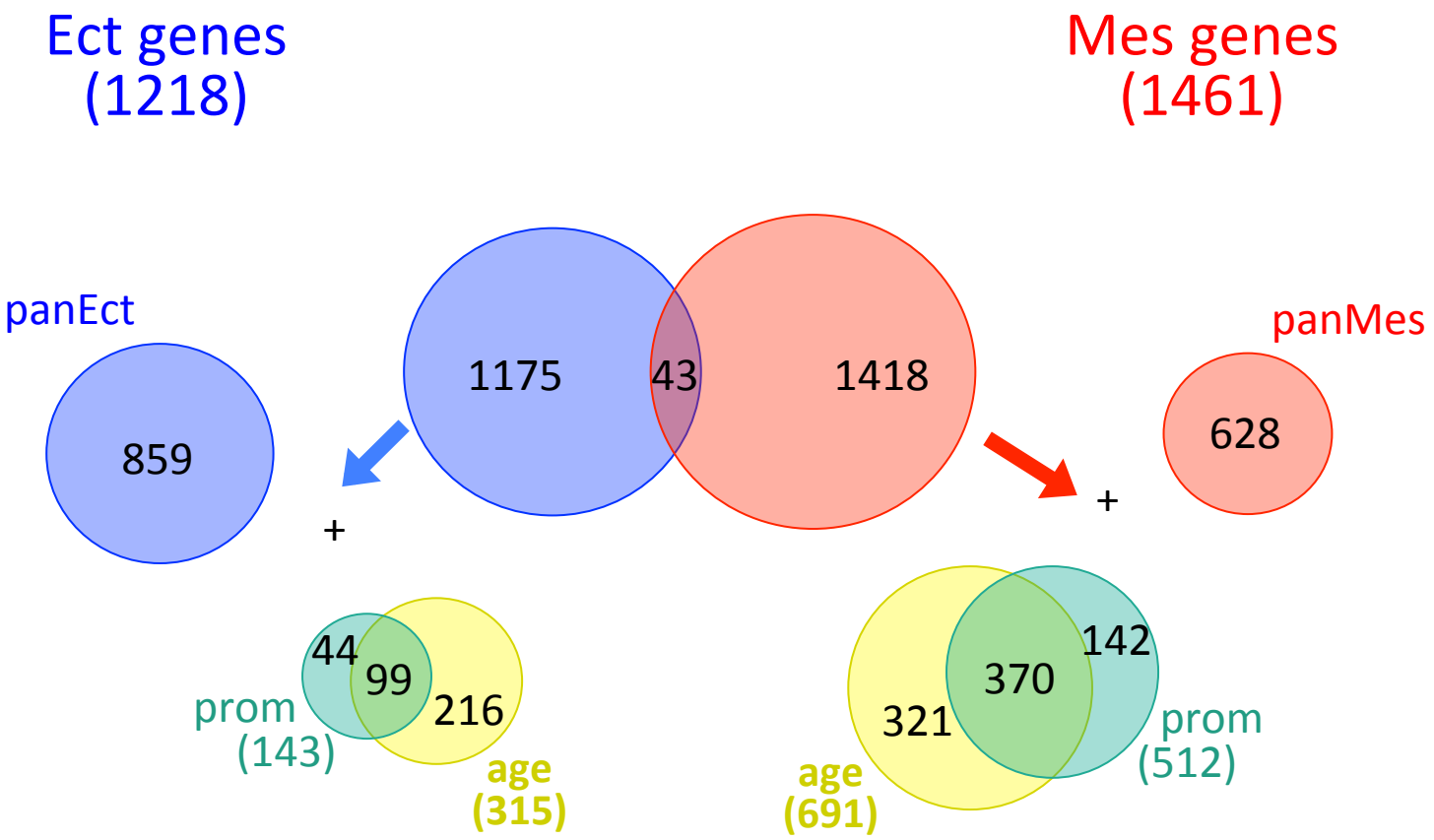
… systems biology of
facial development



Ectoderm-mesenchyme differences dominate

Dissecting the ectodermal program:

1218 genes enriched in ectoderm: 1461 genes enriched in mesenchyme
(fc > 2; p.adj < 0.01)



pan-Ectoderm gene functions

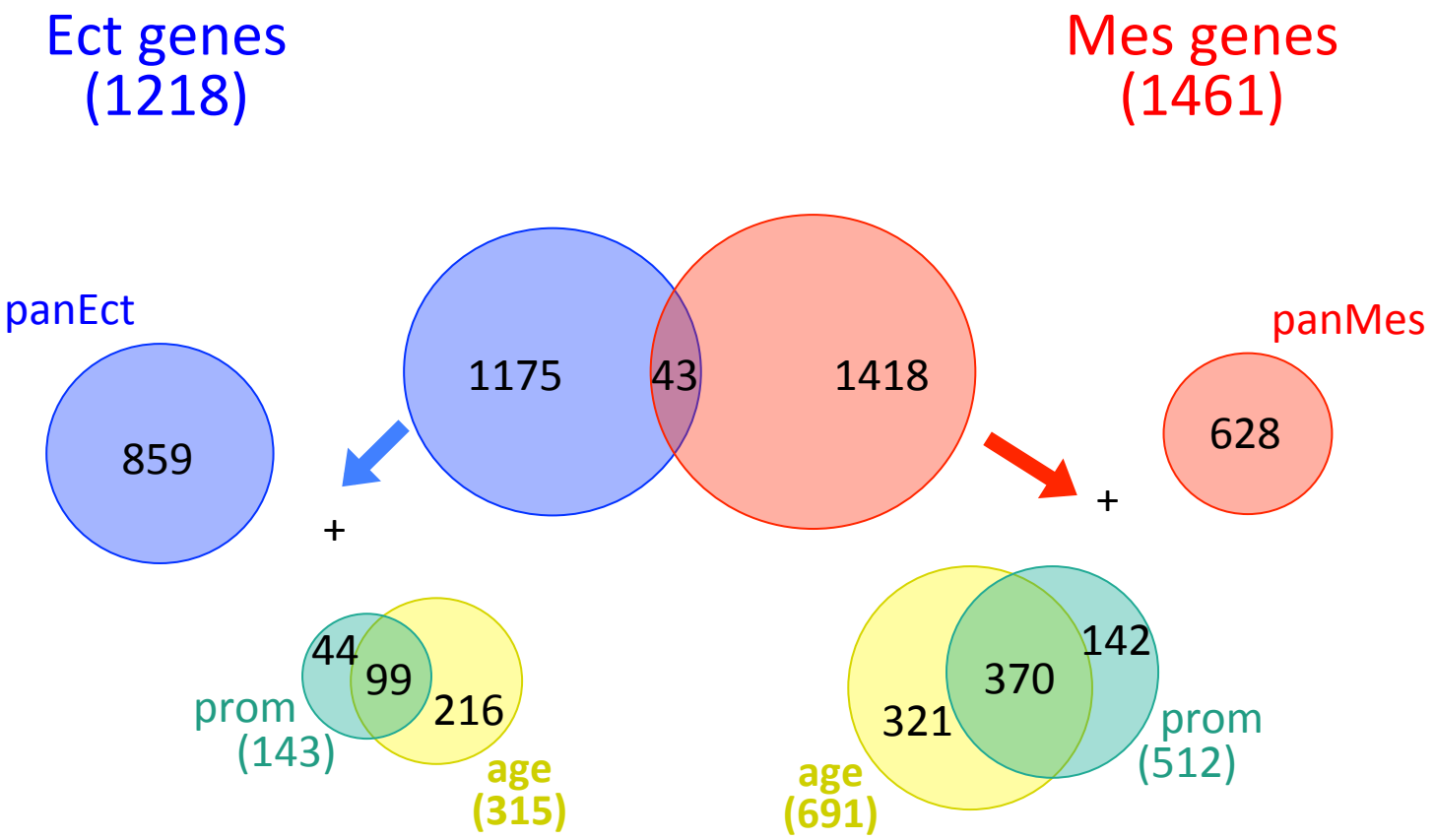
Gene ontology term (gene count)	Enrichment	P.adjust
epithelial morphogenesis & development (104)		
epithelium development (36)	3.3	7.04E-07
tissue morphogenesis (32)	3.3	2.66E-06
tube development (32)	3.0	2.30E-05
gland development (26)	3.3	7.69E-05
morphogenesis of a branching structure (19)	3.8	3.26E-04
cell morphogenesis (32)	2.6	3.26E-04
regulation of cell morphogenesis (16)	4.1	7.43E-04
epithelial cell differentiation (18)	3.6	8.40E-04
morphogenesis of a polarized epithelium (6)	13.5	2.89E-03
epidermis development (17)	3.4	2.88E-03
cell morphogenesis involved in differentiation (21)	2.5	1.57E-02
regulation of morphogenesis of a branching structure (7)	7.0	1.61E-02
keratinocyte proliferation (5)	12.4	1.87E-02
morphogenesis of embryonic epithelium (11)	3.5	3.07E-02
odontogenesis (12)	6.6	1.86E-04
hair follicle development (10)	5.0	7.60E-03
neural development (66)		
regulation of nervous system development (21)	3.5	2.68E-04
neuron differentiation (36)	2.2	1.20E-03
cell projection organization (28)	2.2	1.05E-02
sensory organ development (24)	2.3	1.28E-02
other development (77)		
mesoderm development (10)	4.2	2.00E-02
embryonic morphogenesis (29)	2.0	2.22E-02
embryonic organ development (21)	2.2	4.41E-02
cell fate commitment (16)	2.7	2.62E-02
negative regulation of cell development (8)	5.0	2.85E-02
positive regulation of developmental process (20)	2.3	2.95E-02
vasculature development (22)	2.2	3.22E-02
other (76)		
sulfur metabolic process (12)	3.2	3.56E-02
phosphate metabolic process (54)	1.5	3.72E-02

Gene ontology term (gene count)	Enrichment	P.adjust
adhesion & motility (98)		
cell adhesion (65)	2.9	5.98E-11
cell-cell adhesion (32)	3.4	3.25E-06
regulation of cell adhesion (14)	3.7	5.66E-03
calcium-independent cell-cell adhesion (7)	7.6	1.09E-02
homophilic cell adhesion (15)	3.2	1.15E-02
cell motion (32)	2.2	5.17E-03
cell migration (22)	2.3	2.34E-02
extracellular structure organization (16)	2.7	2.81E-02
signaling (151)		
intracellular signaling cascade (72)	2.0	1.71E-05
regulation of kinase activity (21)	2.7	5.75E-03
enzyme linked receptor protein signaling pathway (26)	2.4	6.25E-03
cell-cell signaling (27)	2.3	6.32E-03
regulation of Ras protein signal transduction (19)	2.6	1.59E-02
negative regulation of signal transduction (18)	2.6	2.02E-02
establishment of planar polarity (4)	19.9	2.17E-02
regulation of phosphorylation (25)	2.1	2.30E-02
transmembrane receptor protein tyrosine kinase signaling pathway (19)	2.5	2.40E-02
positive regulation of catalytic activity (23)	2.2	2.64E-02
Wnt receptor signaling pathway, calcium modulating pathway (6)	7.5	2.84E-02
protein amino acid phosphorylation (43)	1.7	3.27E-02
negative regulation of cell communication (18)	2.4	3.55E-02
Wnt receptor signaling pathway (14)	2.7	5.04E-02
regulation of growth & proliferation (70)		
regulation of cell proliferation (48)	2.2	8.08E-05
negative regulation of cell differentiation (22)	3.0	1.25E-03
regulation of developmental growth (8)	5.4	2.13E-02
regulation of cell growth (12)	3.2	3.13E-02

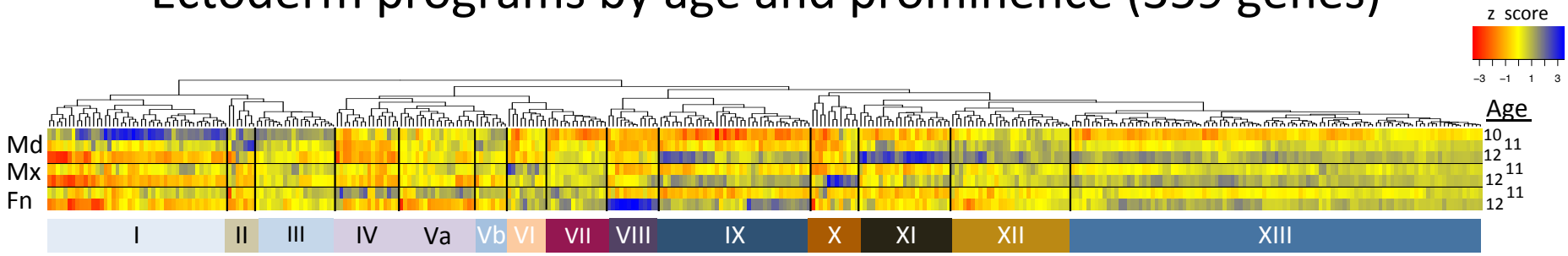
547 of 859 pan-Ectodermal genes were associated with Biological Process GO terms. 310 were associated with enriched terms. Fold enrichment and P.adjust (P-value, with Benjamini adjustment for multiple testing) were determined using DAVID analysis. All enriched terms (p.adjust < 0.05) are reported here, after grouping by genes and eliminating redundant terms.

Dissecting the ectodermal program:

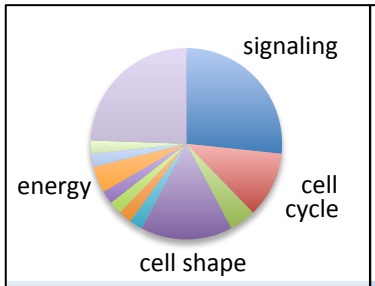
1218 genes enriched in ectoderm: 1461 genes enriched in mesenchyme
(fc > 2; p.adj < 0.01)



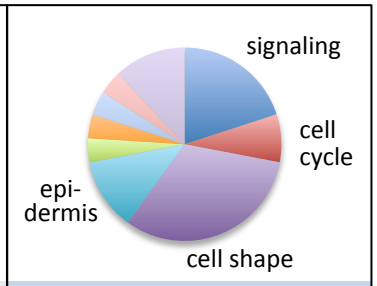
Ectoderm programs by age and prominence (359 genes)



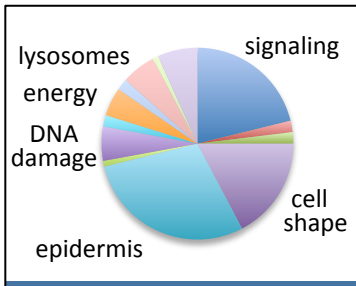
shared



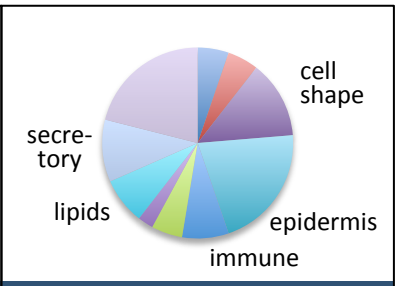
I. early (37)



III+Vb. early-mid (25)

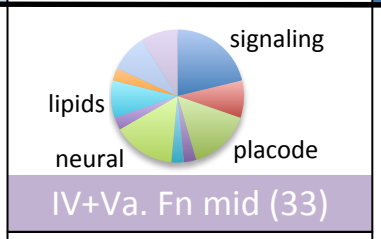


XIII. mid-late (92)

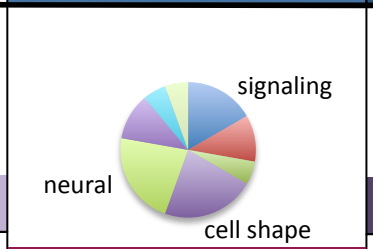


IX. late (35)

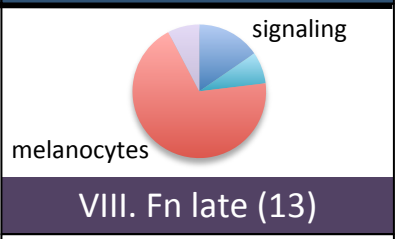
specific



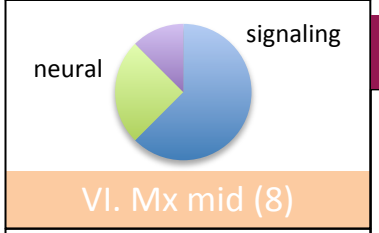
IV+Va. Fn mid (33)



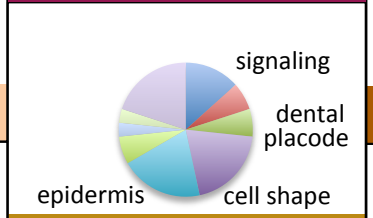
VII. Fn & Mx mid-late (23)



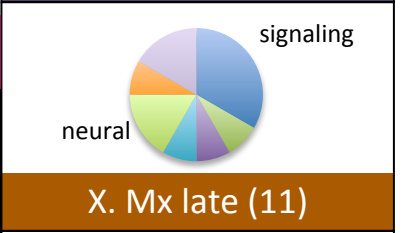
VIII. Fn late (13)



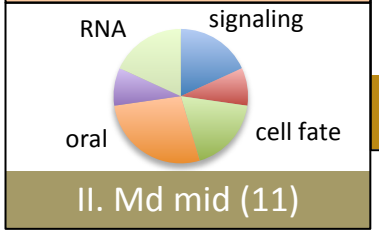
VI. Mx mid (8)



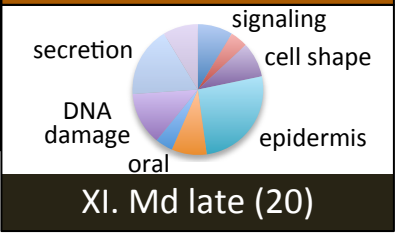
XII. Mx & Md mid-late (26)



X. Mx late (11)



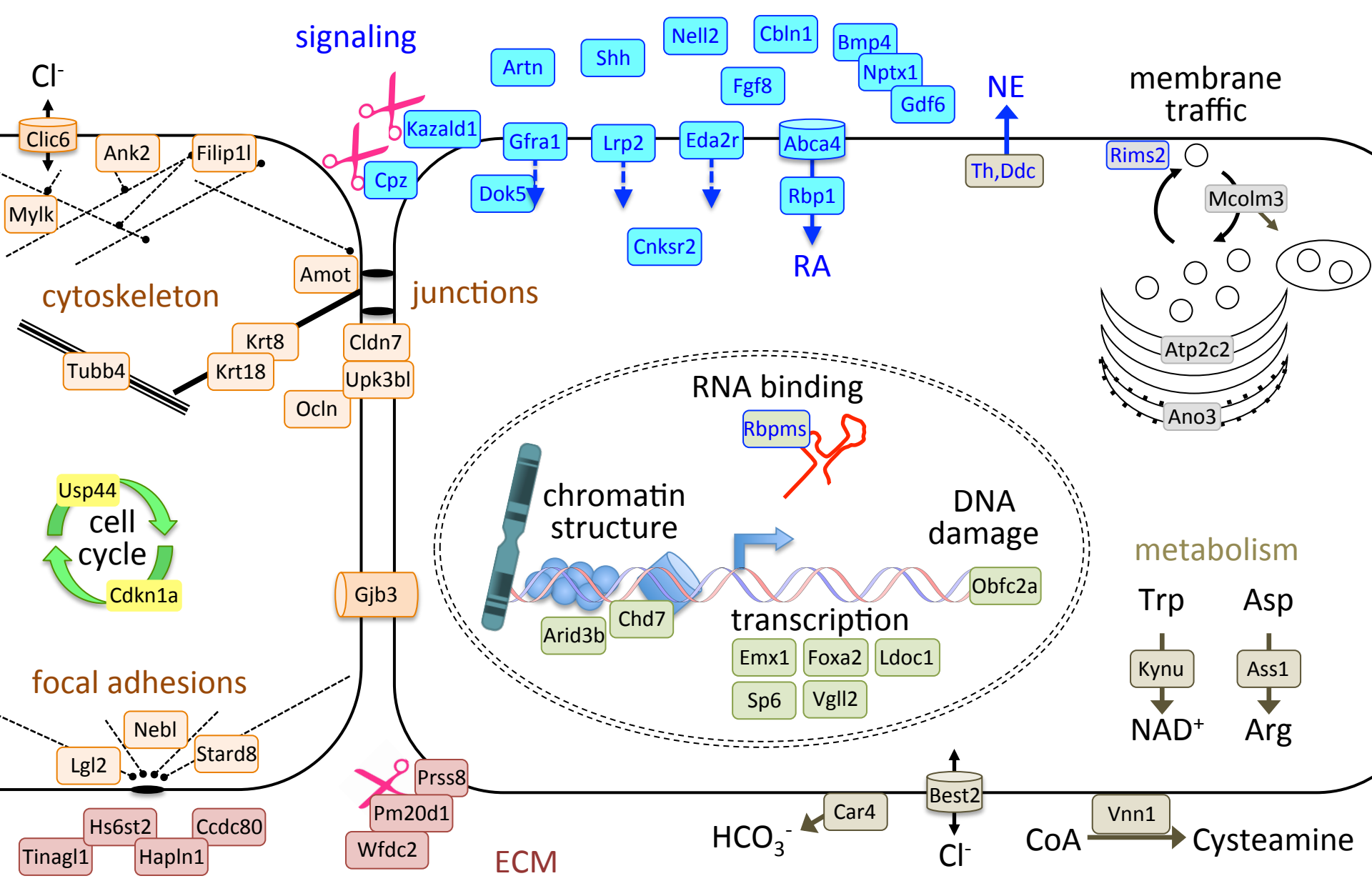
II. Md mid (11)



XI. Md late (20)

Function key

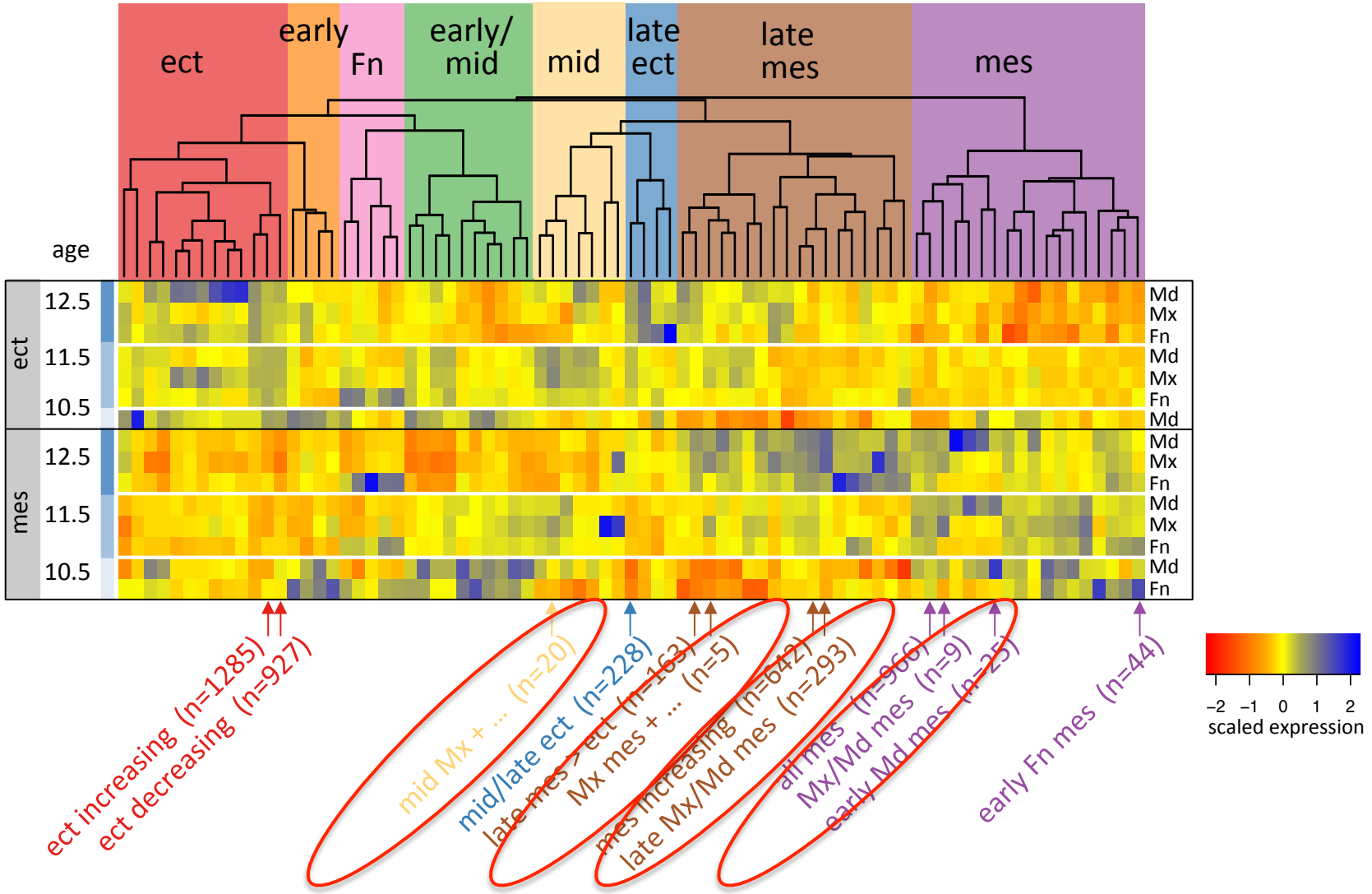
- signaling
- cell fate
- epidermis
- immunity
- neural
- lipids
- secretory
- RNA dynamics
- cell cycle vs. diff
- cell shape
- oral epithelium
- melanocytes
- redox, stress, death
- metabolism
- lysosomes, etc.
- other



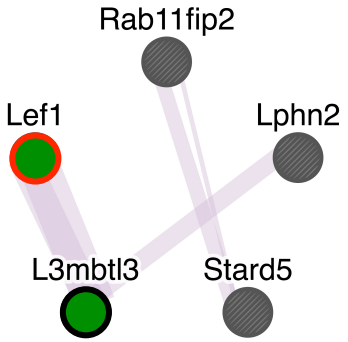
the early ectoderm program

co-expression = shared function

Co-expression modules & clefting



Maxilla clefting modules



Module eigengene expression

Mx mes, etc.

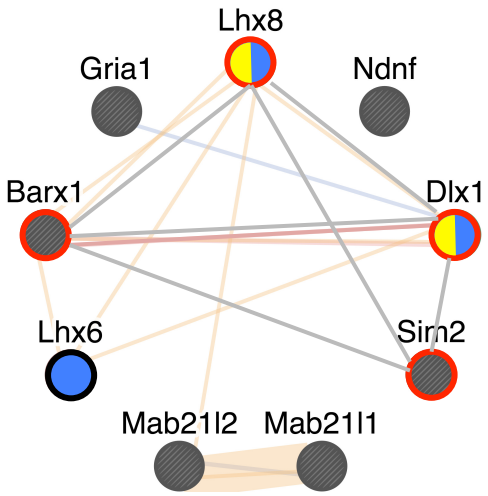
GO BP annotation

granulocyte differentiation

Network connections

Co-expression in GEO

Maxilla clefting modules



Module eigengene expression

Mx & Md mes

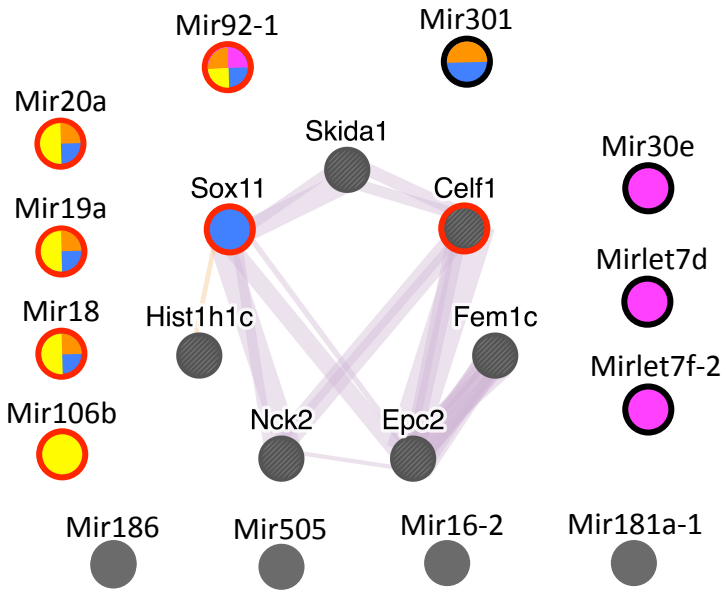
GO BP annotation

- forebrain neuron differentiation
- odontogenesis

Network connections

- Tissue co-localization
- Predicted interaction
- Physical interaction
- Shared phenotype

Maxilla clefting modules



Module eigengene expression

E11.5 mes & ect; Mx, etc.

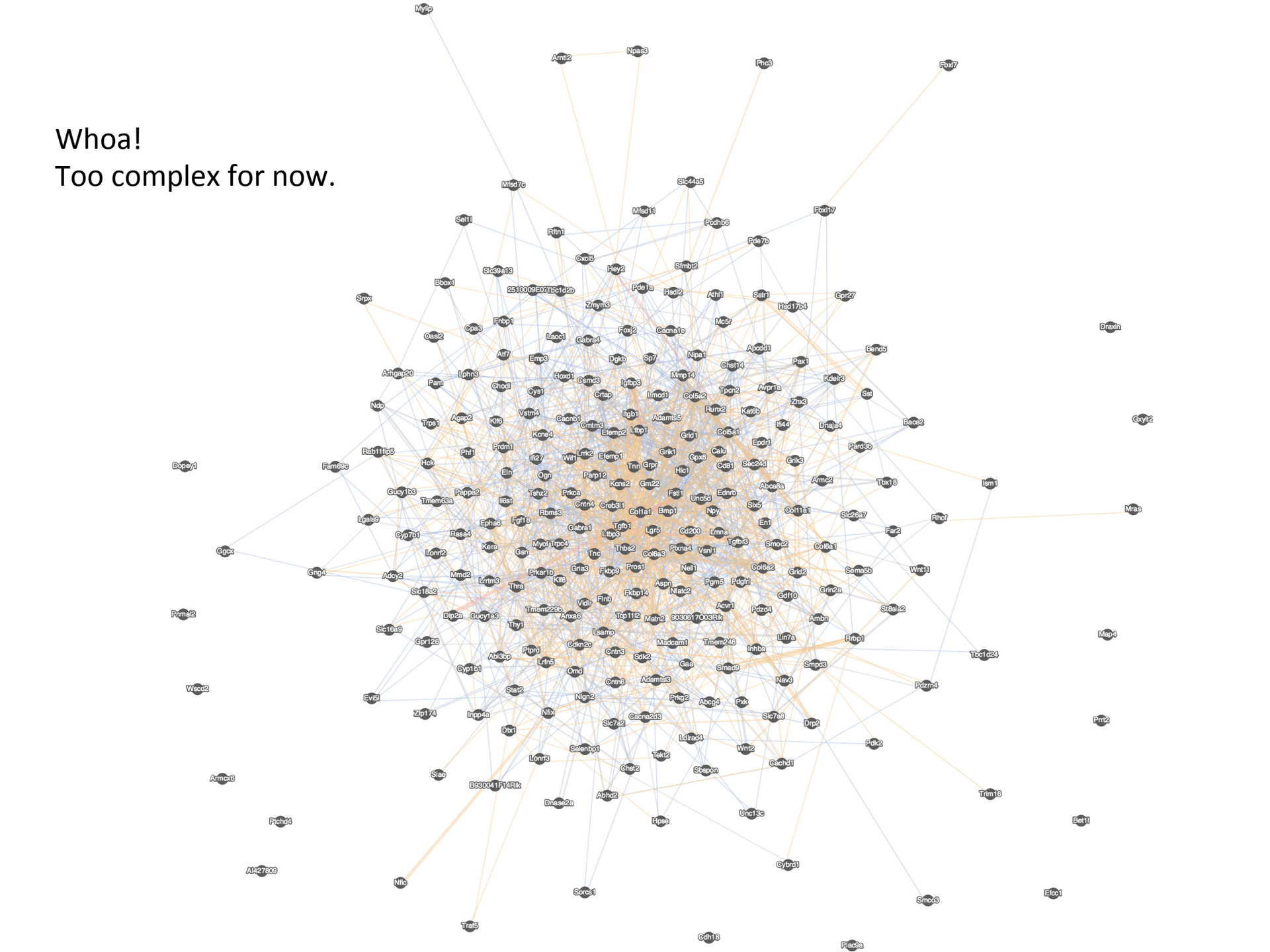
GO BP annotation

- heart morphogenesis
- B cell apoptotic process
- lymphocyte differentiation
- response to amino acid stimulus

Network connections

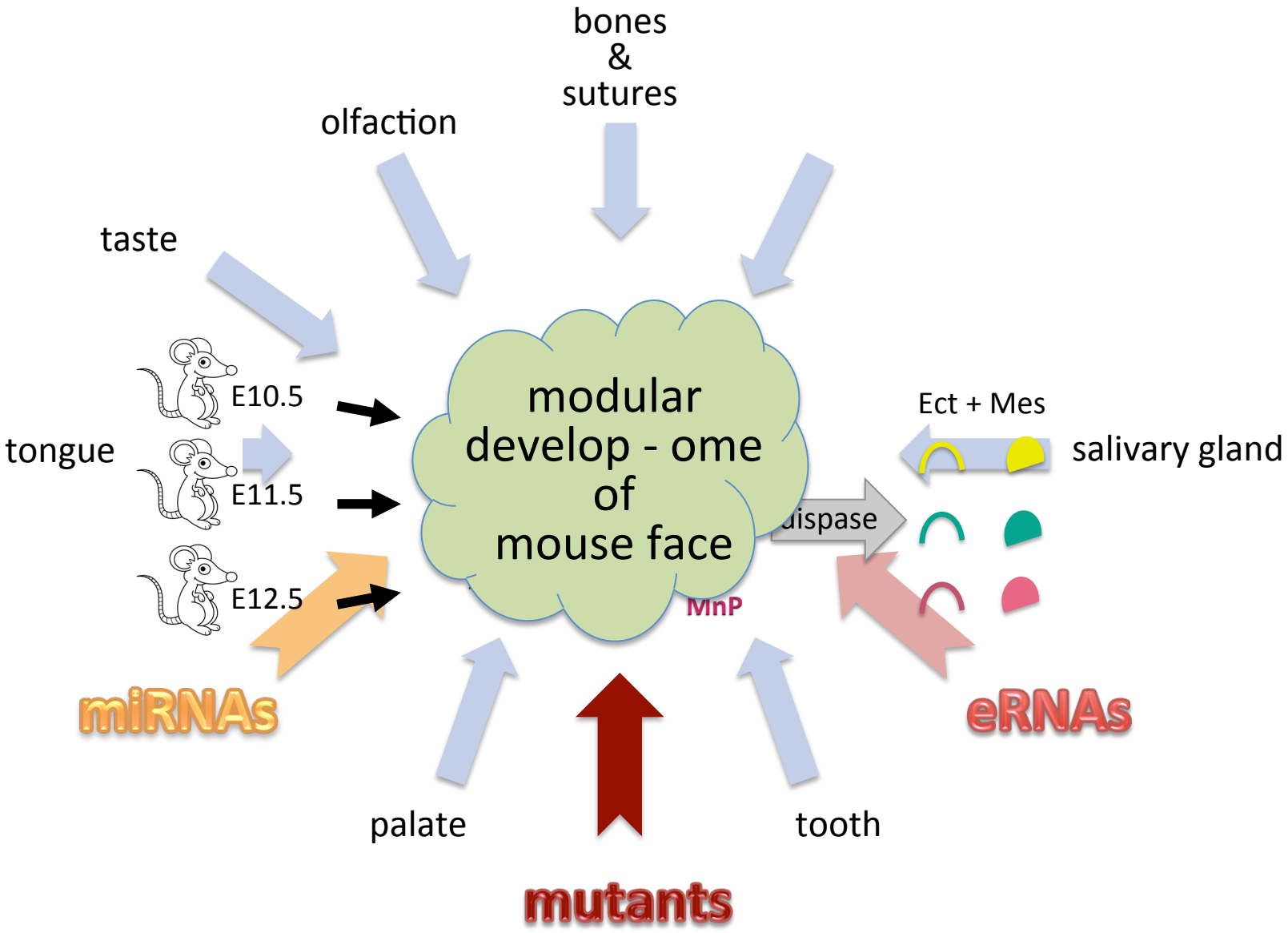
- Co-expression in GEO
- Predicted interaction

Whoa!
Too complex for now.



co-expression modules?

- probably useful to address
  - what groups of genes are in a particular time/ place/tissue?
  - what are the 'funtions' that these groups pinpoint?
  - I've got an 'unknown' or poorly annotated gene. What does it do?


bones
&
sutures
olfaction
taste
E10.5
E11.5
E12.5
tongue
modular
develop - ome
of
mouse face
MnP
Ect + Mes
salivary gland
dispase
miRNAs
eRNAs
palate
tooth
mutants

QUESTIONS?