Cone photoreceptor progenitors in the developing mammalian retina

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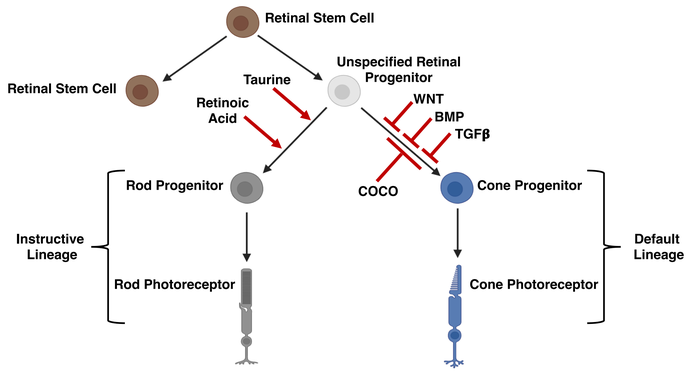
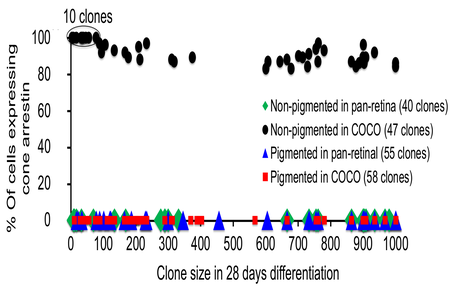
Model of cone photoreceptor lineage specification



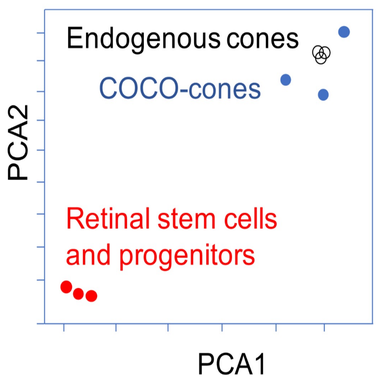
Hypothesis: In the absence of instructive cues from the retinal environment, neural retinal progenitors assume a default fate of cone photoreceptor-restricted lineage.

COCO, a multifactorial inhibitor of BMP, TGFβ, and WNT signaling promotes retinal progenitors to adopt cone-restricted lineage

A. Only non-pigmented neural retinal progenitors derived from adult retinal stem cells display a clonal cone restricted fate



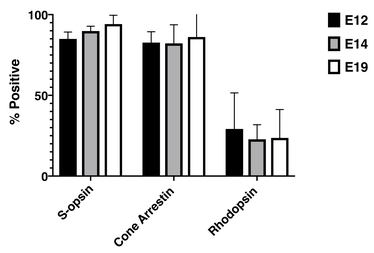
B. Principle component analysis shows that retinal stem cell-derived cones are similar to endogenous adult cones



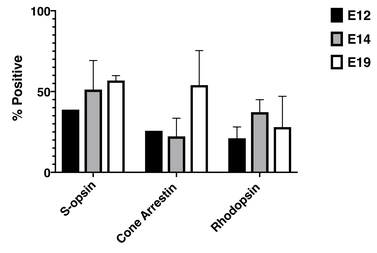
A. Adult murine retinal stem cell (RSC) derived colonies were FACS sorted for pigmented RPE progenitors and non-pigmented neural retinal (NR) progenitors. Sorted cells were then plated at a single cell per well and exposed to the presence or absence (pan-retinal) of COCO for 28 days. Only non-pigmented NR progenitors, when exposed to COCO, produced pure cone photoreceptor clones. There were no significant differences in clone size and survival between the different groups (data not shown). **B.** Principle component analysis of the whole transcriptomes of 28 day differentiated RSC-derived cones, endogenous cones, or RSC-derived colonies

COCO can specify cone restricted fate in retinal progenitors irrespective of developmental age or proliferative capacity

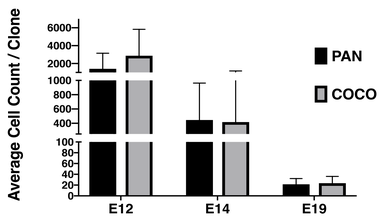
A. Constant COCO exposure causes neural retinal progenitors from all developmental ages to adopt a cone restricted fate



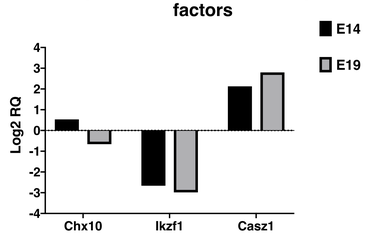
B. In the absence of COCO, neural retinal progenitors do not display nearly pure cone enrichment across developmental time



C. Differentiating neural retinal progenitors show a reduction in colony size with increasing developmental age



D. Neural retinal progenitor colonies maintain the expression of temporal competence factors



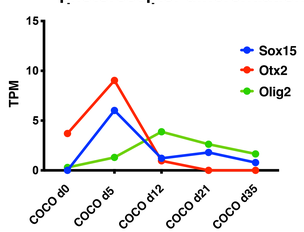
Progenitor cells from multiple embryonic ages were first expanded into clonal sphere colonies and then differentiated in the **A.** presence or **B.** absence (pan) of COCO for 4 weeks. Immunofluorescence analysis was used to quantify the composition of photoreceptors and size (**C.**) of each clone. **D.** Collections of progenitor colonies from different ages were analyzed for expression of early (Ikzf1) vs. late (Casz1) retinal temporal genes relative to E12 progenitors.

RNA-seq analysis across cone differentiation reveals Sox15 as a potential cone progenitor marker

A. Top 10 differentially expressed genes between day 5 cone differentiation and day 0

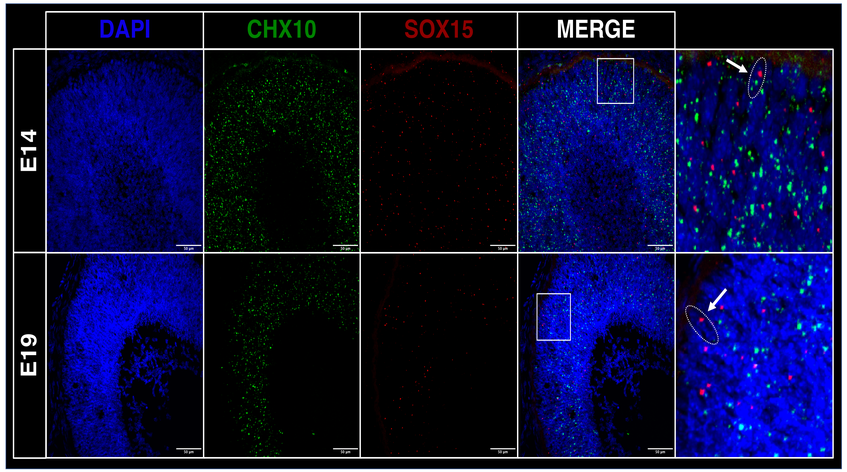
Gene	Function
Itga4 - Integrin subunit alpha 4	Cell adhesion, motility, and migration
CPNE6 - Copine 6	Calcium-dependent, phospholipid-binding proteins. Membrane trafficking and synaptic plasticity.
GLP2R - Glucagon like peptide 2 receptor	GPCR that binds to glucagon-like-peptide-2. Stimulates intestinal growth.
TIGIT – T Cell Immunoreceptor With Ig And ITIM Domains	Family of immunoglobin proteins. Expressed on several classes of T cells includfollicular B helper T cells.
Tspan10 - Tetraspanin 10	Cell surface protein involved in mediating signal transduction events that play a role in the regulation of cell development, activation, growth and motility
CTRL - Chymotrypsin Like	A zymogen expressed specifically in the pancreas and likely functions as a digestive enzyme.
Sox15	Transcription factors involved in the regulation of embryonic development and in the determination of the cell fate.
Reep6 - Receptor Accessory Protein 6	Involved in the transport of receptors from the endoplasmic reticulum (ER) to the cell surface. This gene is required for the proper development of retinal rods and photoreceptors, with defects in this gene being associated with retinitis pigmentosa.
GAS6 - Growth Arrest Specific 6	Encodes a gamma-carboxyglutamic acid (Gla)-containing protein thought to be involved in the stimulation of cell proliferation
Prph2 - Peripherin 2	Photoreceptor outersegment protein

B. Sox15 expression across cone development follows that of other known factors that bias progenitors towards photoreceptor differentiation



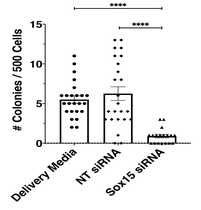
Neural retinal progenitors from E14 retina were differentiated in the presence of COCO and subject to RNA-sequencing across multiple timepoints during cone differentiation. **A.** List of top differentially upregulated genes after five days of COCO exposure. **B.** Normalized expression (TPM) of Sox15 and other photoreceptor precursor markers across the differentiation timecourse.

Sox15 is expressed in a subset of early neural retinal progenitors and photoreceptor precursors *in vivo*

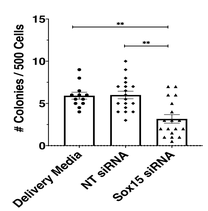


Sox15 may be important for proliferative competency and/or survival of early and late retinal progenitors

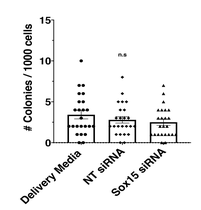
A. E14 RPC Sphere siRNA



B. E19 RPC Sphere siRNA



C. Adult RSC Sphere siRNA



Neural retinal progenitors from E14 (**A.**), E19 (**B.**), or adult retinal stem cells (RSCs) (**C.**) were plated at a clonal density (5 cells/uL) and exposed to Sox15 or non-target control siRNA and allowed to proliferate into free floating sphere colonies for 7 days.

Working models of Sox15 activity

Downstream of COCO, Sox15 promotes cone differentiation through:

Prolonging or promoting proliferation of early retinal progenitors

AND / OR

Inhibiting the activity of transcription factors that specify differentiation to later born retinal cell types

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