



AB040. Pou2f1/2 are required for the specification of cone photoreceptors in the developing retina

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Background: Rods and cones are critical for light detection. Although there has been considerable work done in elucidating the molecular mechanisms involved in rod development, not much is known about how the cone cell fate decision is made by the multipotent retinal progenitor cells during development. Analysis of the promoter regions of *Nrl* and *trβ2*, rod and cone differentiation factors respectively, revealed DNA binding motifs of two POU-domain containing transcription factors, *Pou2f1* and *Pou2f2*. Preliminary experiments showed that *Pou2f1/2* are expressed during the peak of cone genesis in the embryonic retina. Therefore, we hypothesize that *Pou2f1/2* specify cone cell fate in the developing retina.

Methods: We used immunofluorescence and *in situ* hybridization to establish the spatiotemporal expression of *Pou2f1/2* during retinogenesis. We performed *in vivo* electroporation in post-natal mice to misexpress *Pou2f1/2* and used antibodies specific to proteins expressed in cones such as *Rxry* and S-opsin to count cones. Using *ex vivo* electroporation of embryonic retinal explants, we knocked out *Pou2f1* and *Pou2f2* using CRISPR/Cas9 gRNAs at the peak of cone production window. Finally, we transfected post-natal retinal explants with a combination of regulatory elements of *Nrl* or *thrb* with control backbone vector, *Pou2f1* or *Pou2f2* using electroporation.

Results: We found that *Pou2f1/2* are expressed in retinal progenitor cells in the developing retina and subsequently in the differentiated cones. *Pou2f1/2* misexpression outside the cone genesis window led to an increase in cones at the expense of rods. *Pou2f1/2* indel knockouts generated by CRISPR/Cas9 gRNAs led to a decrease in cones and a converse increase in rods. Finally, we found that *Pou2f1/2* activate the cis-regulatory module (CRM) of the *thrb* gene and repress the activity of the CRM of *Nrl*.

Conclusions: These results uncover novel players that establish the complex gene regulatory network for cone photoreceptor fate specification in the retinal progenitor cells. We anticipate that this work should help us devise improved replacement therapies in the future utilizing stem cells for retinal degenerative diseases such as aged-related macular degeneration (AMD) and Stargardt's disease.

Keywords: Cones; rods; cell biology; molecular biology; retinogenesis

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