subset of cells in the INL, and is gradually re-expressed in all rod photoreceptors. During retinal regeneration, in situ hybridization reveals that neuroD is not expressed in newly formed progenitor cells, but is expressed in progenitor cells as they migrate toward the ONL and differentiate into new rod photoreceptors. The nrd:egfp transgene shows a similar expression pattern during this dynamic regenerative process. We conclude that Tg(nrd:egfp) is a good representation of endogenous neuroD expression, and will be a useful tool in understanding the fundamental processes behind retinal development and regeneration.

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Program/Abstract #389
Expression of the Eph/ephrin system in chick retina regeneration
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The embryonic chick has the capacity to regenerate its retina upon retinectomy via the activation of stem/progenitor cells in the anterior margin of the eye or by the transdifferentiation of the retinal pigmented epithelium. The optimal outcome of retinal regeneration is to obtain a functional retina. In this context, the Retinal Ganglion Cell (RGC) axons must reach the optic tectum (OT) and form the correct topographic map. Ephs and ephrins constitute the main molecular system involved in this process. The Eph receptor family consists of a group of transmembrane protein kinases that include two subgroups, the EphA and EphB receptors, depending on the ligands with which they interact. Ephrins can bind Eph receptors via their receptor binding domain. Ephrin-As are anchored to the membrane by Glycosylphosphatidylinositol whereas ephrin-Bs are transmembrane proteins. The Ephs and the ephrins are expressed in opposite gradients in the developing retina and the OT. The EphA/ephrinA system is involved in defining the topographic retinotectal connections along the rostrocaudal axis, whereas the EphB/ephrinB system is implicated in the dorsoventral axis and also in directing RGC axon path finding from their position in the retina to the Optic Disc (OD). In this study we analyze the expression of the Eph/ephrin system in the developing and regenerating chick retina in order to establish if the miss-expression of this system could be one of the reasons for the failure of the RGC axons of the transdifferentiated retina to reach the OD. For this purpose we performed immunohisto-chemistry against several Ephs and ephrins in developing and regenerated retinas at different developmental stages. Funded by CONICET and Miami University.

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Program/Abstract #390
Expression of stem cell pluripotency-inducing factors during chick retina regeneration
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The embryonic chicken can regenerate its retina by the reprogramming of the retinal pigmented epithelium (RPE) and by the activation of stem/progenitor cells present in the Ciliary Margin (CM) located in the anterior region of the eye. It has been demonstrated that somatic cells can be reprogrammed in vitro to generate induced pluripotent stem cells (iPSC) by expression of Oct4, Sox2, cMyc and Klf4. However, there is limited information concerning the reprogramming during the process of regeneration in vivo. Here, we test the hypothesis that reprogramming of the RPE share similarities to the reprogramming of somatic cells that generate iPSC. Therefore, we analyzed the expression of stem cell pluripotency factors during chick retina regeneration. We first collected CM and RPE by Laser Capture Microdissection (LCM) from embryos at day 4–7. Among all factors, only Sox2, cMyc and Klf4 mRNAs were detected by RT-PCR in the CM. Sox2 was clearly detected by immunofluorescence in the CM and central retina but not in the RPE. In contrast, only cMyc mRNA was detected in the RPE. During retina regeneration, Sox2, cMyc and Klf4 remained expressed in the CM. However, Sox2 and Klf4 were induced after 4 h post-retinectomy during RPE reprogramming, and their expression was maintained up to day 3 in the presence of FGF. Interestingly, Sox2 was immediately up-regulated in the RPE at 4 h post-retinectomy even in the absence of FGF but its expression is completely lost at day 3 post-retinectomy. These results suggest that FGF is necessary to maintain the expression of Sox2 during the RPE reprogramming. Oct4 and Nanog were not detected during regeneration indicating that reprogrammed RPE cells do not generate pluripotent cells.

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Program/Abstract #391
Canonical Shh signaling inhibits FGF-induced transdifferentiation
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The embryonic chick is able to regenerate its retina after complete removal through the transdifferentiation of the Retinal Pigmented Epithelium (RPE) if Fibroblast Growth Factor 2 (FGF2) is present. In this process, the RPE cells dedifferentiate, losing their features, proliferating and differentiating into retina. Our lab has shown that overexpression of Sonic Hedgehog stops FGF-induced retina regeneration. This study examines the mechanisms by which Sonic Hedgehog (Shh) inhibits FGF-induced transdifferentiation. To address this question, we over-expressed Gli-1 (a downstream target molecule of Shh) in embryonic chick eyes undergoing FGF-induced regeneration, to evaluate if Sonic Hedgehog is acting through its canonical pathway. Chick eyes at embryonic day 3.5 (E3.5) were injected subretinally with an RCAS virus containing Gli-1 (RCAS-Gli-1). At E4 retinectomies were performed and FGF2 beads were introduced into eye cups to stimulate regeneration. Eyes were collected three days after surgery, fixed and tested by immunohistochemistry for RPE and retina markers, or stained for histology. Immunohistochemical studies show that Gli-1 upregulates RPE markers such as Mift and Otx-2 as well as p27, a negative regulator of the cell cycle, keeping the fate of RPE. This treatment also down regulates Pax-6, an essential molecule for retina regeneration. At histological level, the overexpression of Gli-1 inhibited RPE transdifferentiation. In conclusion, the results suggest that Shh can inhibit FGF-induced transdifferentiation through its canonical pathway via Gli-1, maintaining the identity of RPE and keeping RPE cells in cell cycle arrest. Supported by NIH EY017319-05 and CONACYT.

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