Introduction

Axons are actively guided to their targets in the nervous system during development by chemoattractive and chemorepulsive guidance cues. Slit2, signaling via the receptor Robo2, is a powerful chemorepulsive cue for retinal ganglion cell (RGC) axons (Nicu et al. 2000, Fricke et al. 2001). The chemokine SDF-1, binding to its receptor CXCR4, activates an ‘anti-repellent’ signaling pathway that antagonizes the repellent activity of Slit2 (Chalasani et al. 2003). In a bioassay for repellent activity, when SDF-1 is present, Slit2 is eight times less effective as a repellent (Chalasani et al. 2003).

SDF-1 signaling has been shown to elevate cAMP levels; therefore, it is reasonable to hypothesize that cAMP synthetizing enzymes, the adenylyl cyclases (ADCYs), are activated by SDF-1 (Chalasani et al. 2003). Furthermore, blocking calcium/calmodulin has been shown to interfere with SDF-1 signaling (Chalasani et al. 2003).

I hypothesized that the Ca²⁺/calmodulin-activated ADCY8 is a necessary component of the SDF-1 anti-repellent signaling pathway. I therefore predicted that knock down of ADCY8 would prevent SDF-1 antagonism of Slit2 repellent signaling.

Methods

Morpholinos: Zebrafish embryos were collected and the yolk was injected at the one cell stage with either a control morpholino (MO) or two MOs that target separate intron-exon borders in the ADCY8 pro-RNA. The ADCY8 MOs block splicing and induce premature stop codons in the mRNA.

Zebrafish retinal cultures: Retinal explants were prepared from 2 dpf ADCY8 MO or Control MO injected zebrafish and cultured in supplemented L15 media on laminin (80 µg/ml) and poly-lysine (200 µg/ml) coated MatTek dishes. The explants were cultured for approximately 24 hours at 28.5 degrees Celsius.

Protein preparation: Human Slit2 and Zebrafish SDF-1a were made as secreted proteins by a stable 293T cell line or transfected 293T cells. Crude supernatants containing the proteins were collected and protein content was confirmed by Western blot analysis.

Live imaging: Control MO and ADCY8 MO containing axons were maintained on the heated stage of an inverted microscope. At the start of the experiment and after 75 minutes they were photographed using phase optics. Either 125 µl HSii2, 250 µl zSDF-1a, or 125 µl HSii2 plus 250 µl zSDF-1a were added to the media. Photos were taken after another 75 minutes elapsed. The extension of individual axons during the initial control period and the treated period was compared between conditions.

Results

1. Previous experiments show that SDF-1 signaling antagonizes axonal response to the repellent Slit2, in vitro and in vivo.

2. Retinal axons containing Control MO or ADCY8 MO extend similar distances with or without SDF-1 treatment.

3. Knock down of ADCY8 does not interfere with axon responsiveness to the repellent Slit2.

4. Knock down of ADCY8 blocks SDF-1 antagonism of the Slit2 repellent pathway.

5. Knock down of ADCY8 perturbs retinal axon crossing of the midline.

Literature Cited


Conclusions

• These results are consistent with our prediction that ADCY8 is a necessary component of the SDF-1 pathway that antagonizes Slit2 repellent activity.
• ADCY8 MO containing retinal axons are able to extend normally compared to Control MO axons.
• Knocking down ADCY8 has no effect on axonal response to the repellent Slit2.
• Morpholinos against ADCY8 cause zebrafish to develop with several retinal axon guidance errors including intermittent failure to cross the midline and aberrant projection to the ipsilateral rather than contralateral tectum (Xu and Raper, unpublished).
• We hypothesize that midline guidance errors in the developing fish are caused by hyper-sensitivity of retinal axons to midline repellents.