Morphogenesis and axis specification occur in parallel during optic cup and optic fissure formation, differentially modulated by BMP and Wnt.

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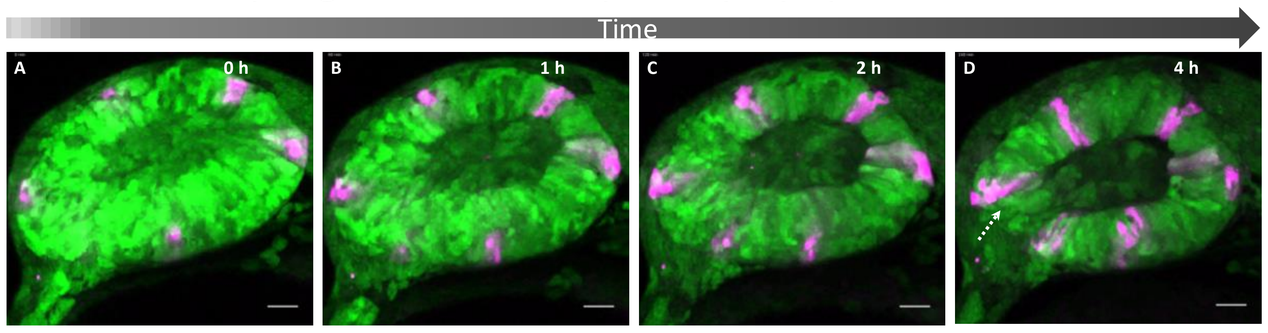
During transformation from the optic vesicle into the optic cup, the optic fissure is formed in the ventral part of the optic cup. It is a transient gap, serving as entry route for tissue and embryonic blood vessels. It closes as development proceeds. If closure fails, coloboma, showing a wide range of morphological phenotypes, occur. In a previous analysis, we observed that optic cup morphogenesis is a highly dynamic process. Here, we aimed to elucidate to what extent the optic fissure is affected by these tissue dynamics to shed more light onto "morphogenetic coloboma".

We addressed optic fissure morphogenesis by 4D *in vivo* time-lapse analyses using zebrafish (*Danio rerio*) as a model. We furthermore studied the role of BMP4-, and Wnt-signaling during optic cup morphogenesis and addressed dorsal-ventral axis specification by whole mount *in situ* hybridization.

We observe that under normal conditions, at the temporal side, the tissue flow that drives optic cup morphogenesis is translating into a ventral flow, shaping the temporal fissure margin. Nasally however, a tissue flow from the optic stalk is largely shaping the fissure margin. Furthermore, we find that both induction of BMP expression, as well as inhibition of Wnt signaling, hamper optic fissure morphogenesis and affect dorsal-ventral axis specification/ maintenance, respectively.

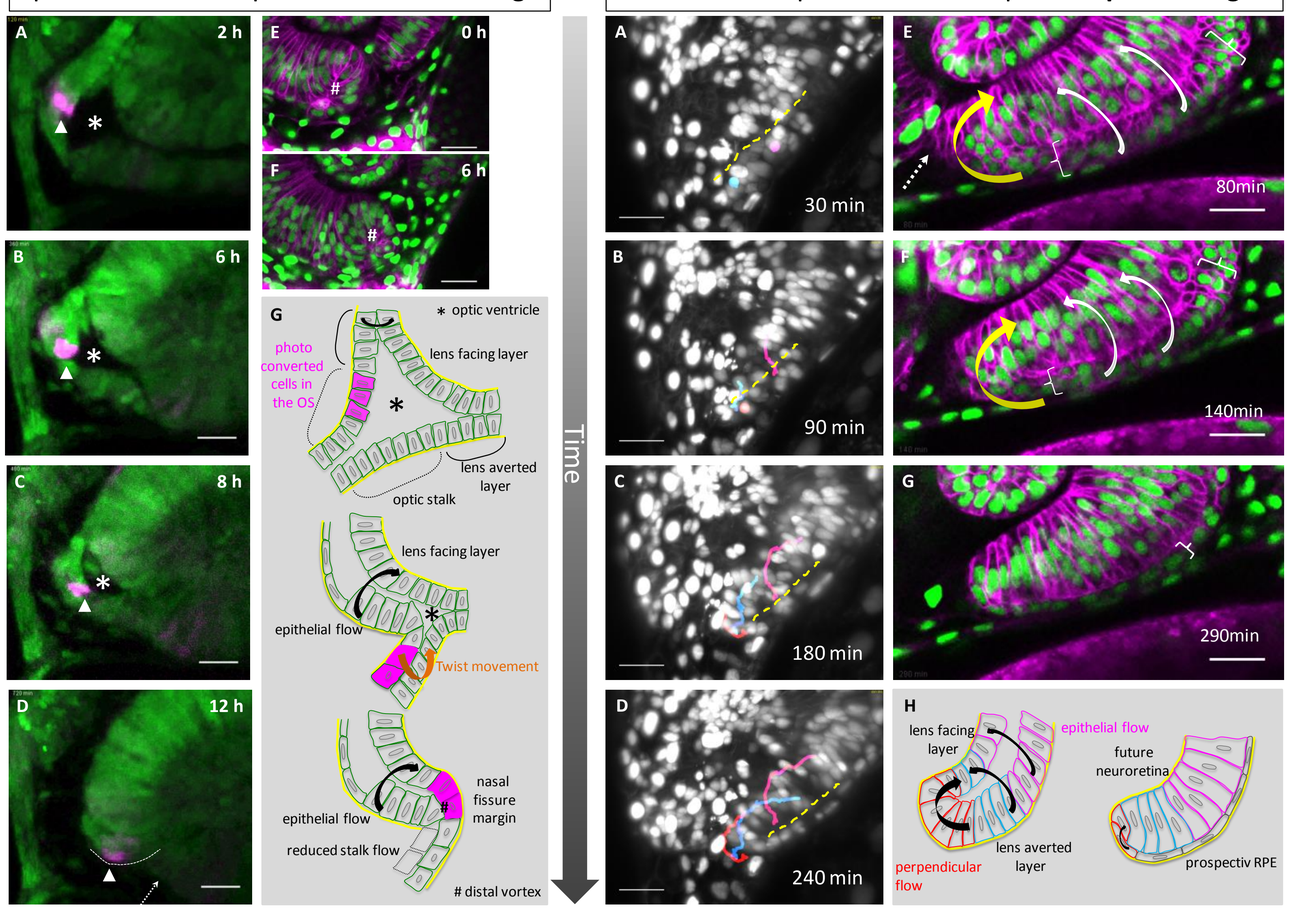
Our analyses show that induced BMP expression and Wnt inhibition both result in morphogenetic coloboma, also affecting the dorsal-ventral axis of the eye. Our data furthermore indicate that optic cup morphogenesis is crucial for a proper positioning of pre-specified dorsal-ventral optic cup domains.

Morphogenesis of the Optic Cup by Epithelial Flow



During optic cup morphogenesis cells from the lens averted layer (A magenta) of the optic cup move over distal rims, in a gastrulation like manner to the lens facing layer (B-D, magenta). During that process the optic fissure is formed (D dotted arrow).

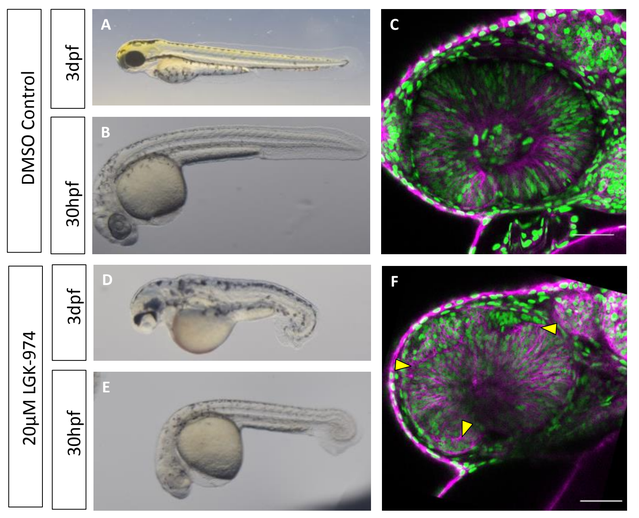
Morphogenesis of the Optic Fissure



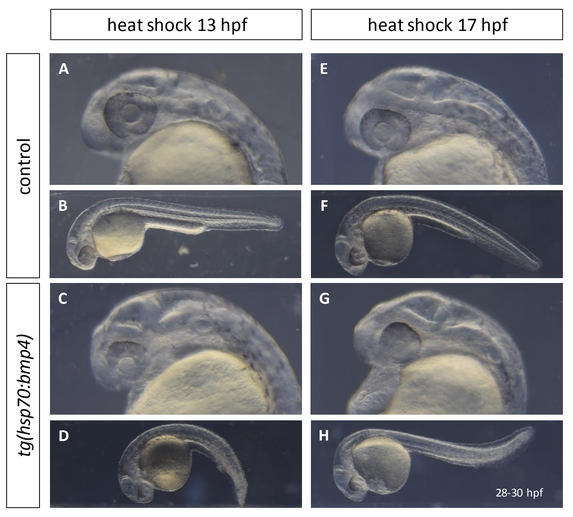
Nasally photo converted cells (magenta cells, arrowhead A-D) from the optic stalk can be followed into the nasal fissure margin (D, dotted line). Distally the stalk flow converges with the epithelial flow, a vortex is formed (E-F #). Scheme of nasal fissure margin development (G)

Temporally the epithelial flow (A-D magenta/ blue tracks, E-F white arrows) from the lens averted layer (E-G white brackets) to the lens facing layer is ventrally translating into a perpendicular flow (A-D red track, E-F yellow arrow). The border between the lens averted and the lens facing layer is indicated with a yellow dashed line (A-D) Scheme of nasal fissure margin development (H)

Zebrafish development overall and eye development is affected by **Inhibition of Wnt secretion** and **Bmp4 overexpression**

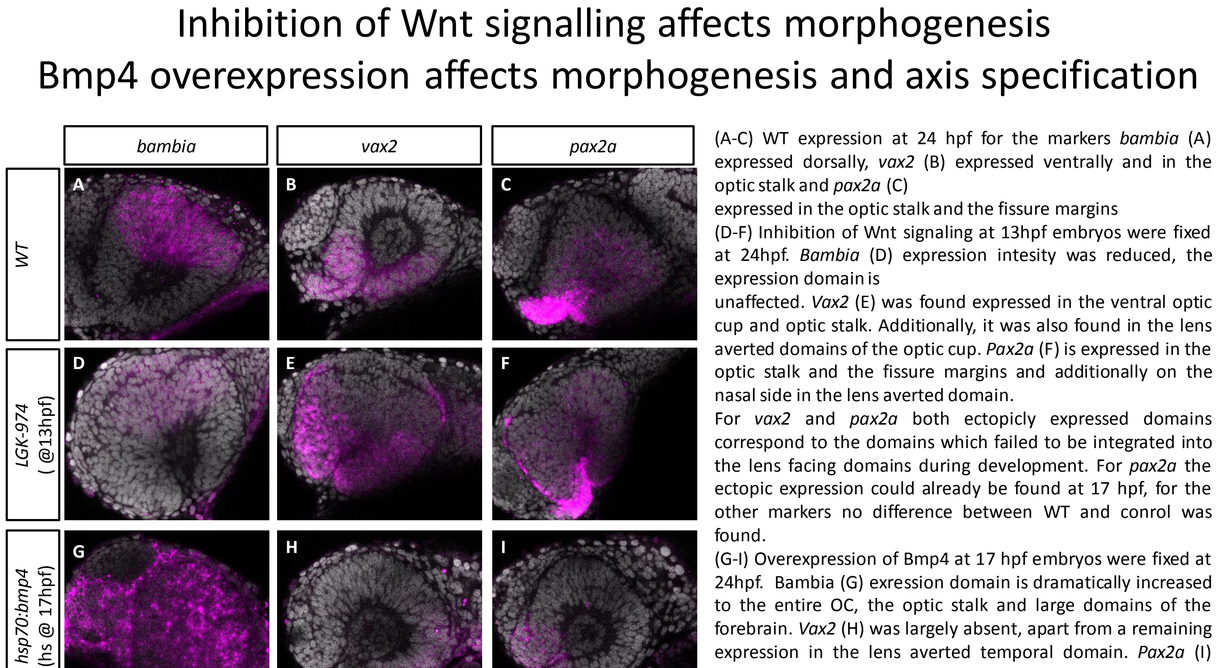


Gross morphological overview and confocal images of Wnt secretion inhibited embryos.
(A-C) DMSO control, (D-F) 20µM LGK-947 in DMSO. Macroscopic pictures were taken at 30hpf (B,E), and 3dpf (A,D). For treated embryos the tail is bent, and the embryo is shortened. In 3dpf fish the RPE is only present in a stripe from proximal towards temporal and nasal, but not dorsal and ventral. Confocal images (C,F) of embryos were taken at 30hpf. The border between the lens-facing neuroretina and ectopic neuroretina in the dorsal area of the eye and the nasal ventral part are marked with yellow arrowheads.



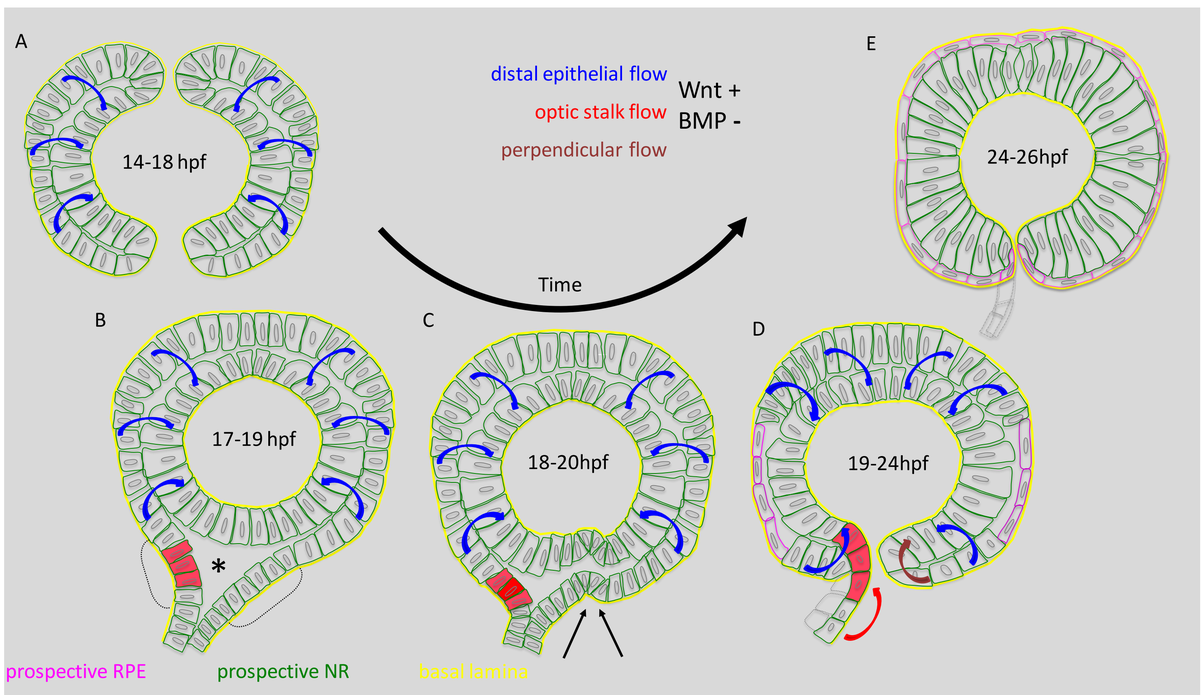
Gross morphological overview of tg*(hsp70:bmp4)* embryos and wild type siblings at age 28-30 hpf. Embryos were heat shocked at 13 hfp (A-D) or 17 hpf (E-H) respectively. In both conditions, the most pronounced defects are seen in eye morphology of BMP overexpressing embryos (C, G). Especially after 13 hpf heat shock, tail growth is also affected (D). The yolk extension is malformed in both conditions (D, H).

Optic Fissure morphogenesis is affected by Inhibition of Wnt secretion and Bmp4 overexpression



In embryos with inhibited Wnt secretion at 13 hpf, TGFβ signaling positive cells are located in the mis-shaped optic stalk/forebrain (A-C arrowheads). Few TGFβ signaling positive cells reach the nasal ventral part of the developing optic cup (C, arrow). The dorsal fissure (A,B marked with v) seems to close over time. Distally the optic fissure is wide open (D)
In embryos with HS induced bmp4 overexpression @ 17hpf, the OF is proximal not forming. The optic stalk is in continuation to the lens-averted domains of the developing optic cup (E-G arrowhead). TGFβ signaling is activated in the OC without tissue dynamics. Distal the optic fissure is wide open (H).
In WT (I-L), TGFβ signaling positive cells move from the optic stalk (I, arrowhead) into the optic cup. The optic stalk is predominantly connected to the nasal fissure margin (I, J). TGFβ signaling positive cells populate the future optic nerve head region, the most proximal domain of the optic fissure (J,K). From here these cells also populate the temporal fissure margin (K white arrow). Distally the margins of the fissure are in close proximity to each other (L). Scalebar: 25µm

Summary: Optic Fissure Morphogenesis



- For morphogenesis of the optic fissure, 3 distinct tissue movements need to play together.
  - the distal flow (blue arrows), that translates only temporal into
  - a perpendicular flow (brown arrow)
  - the stalk flow (red arrow), which brings cells (red color) from the optic stalk into the optic cup.
- Inhibition of Wnt secretion results in:
  - a hampered stalk flow, (TGFb positive cells do not enter the eye)
  - a disturbed neuroretinal flow

  → leading to a phenotype with a short and wide open fissure with ectopic neuroretina.
- Overexpression of Bmp4 during the fissure morphogenesis results in
  - a disturbed stalk flow
  - a disturbed perpendicular flow

  → leading to a phenotype with only a distal fissure (HS @17hpf) or no fissure (HS @ 13hpf)

Inhibition of Wnt signalling affects morphogenesis Bmp4 overexpression affects morphogenesis and axis specification

(A-C) WT expression at 24 hpf for the markers *bambia* (A) expressed dorsally, *vax2* (B) expressed ventrally and in the optic stalk and *pax2a* (C)
expressed in the optic stalk and the fissure margins
(D-F) Inhibition of Wnt signaling at 13hpf embryos were fixed at 24hpf. *Bambia* (D) expression intesity was reduced, the expression domain is
unaffected. *Vax2* (E) was found expressed in the ventral optic cup and optic stalk. Additionally, it was also found in the lens averted domains of the optic cup. *Pax2a* (F) is expressed in the optic stalk and the fissure margins and additionally on the nasal side in the lens averted domain.
For *vax2* and *pax2a* both ectopicly expressed domains correspond to the domains which failed to be integrated into the lens facing domains during development. For *pax2a* the ectopic expression could already be found at 17 hpf, for the other markers no difference between WT and conrol was found.
(G-I) Overexpression of Bmp4 at 17 hpf embryos were fixed at 24hpf. Bambia (G) exression domain is dramatically increased to the entire OC, the optic stalk and large domains of the forebrain. *Vax2* (H) was largely absent, apart from a remaining expression in the lens averted temporal domain. *Pax2a* (I) expression in the fissure margin was also reduced, the domain in the optic stalk, however was found enlarged.

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