

# Involvement of cell polarity regulators in tissue morphogenesis of the zebrafish posterior lateral line organ

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# Abbreviations:

ala	alanine
ATP	adenosine triphosphate
bp	basepairs
C	celsius
CTP	cytidine triphosphate
D	asparagin
ddH <sub>2</sub> O	double distilled water
DIG	digoxygenin
dNTP	deoxynucleotide triphosphate
dUTP	deoxyuridine triphosphate
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
g	gram
glu	glutamate
GFP	green fluorescent protein
GTP	guanosine triphosphate
HCL	hydrogen chloride
hrs	hours
KCL	potassium chloride
kDa	kiloDalton
l	liter
mg	milligram
Mg Cl <sub>2</sub>	magnesium chloride
min	minute
ml	milliliter
mM	millimole
MO	Morpholino
mRNA	messenger ribonucleic acid
NaCl	sodium chloride
NaOH	sodiumhydroxide
ng	nanogram
nl	nanoliter
OD	optical density
pH	potential of hydrogen
RNA	ribonucleic acid
RT	room temperature

S	serine
SDS	sodium dodecyl sulfate
sec	second
ser	serine
Taq	<i>thermos aquaticus</i>
Tris	trishydroxymethylaminomethane
U	unit
UTP	uridine triphosphate
UV	ultraviolet
W	trypsin
WT	wildtype
μg	microgram
μl	microliter
μM	micromolar

# Summary

Tissue morphogenesis and cell sorting are major forces during organ development. It is a puzzling problem, to identify the cellular machineries that underlie cell-cell rearrangements within migratory tissues and during organ morphogenesis. In my work, I focused on a simple migratory tissue, the pll<sub>p</sub> of the zebrafish, as a model to characterize the molecular and cellular mechanisms involved in tissue morphogenesis. During migration, the pll<sub>p</sub> deposits clusters of cells, which will later become the sensory units of the lateral line organ. I show here, that the zebrafish pll<sub>p</sub> is a migratory epithelial tissue. Within this tissue, actin-rich apical adhesion complexes arrange from an alignment with the direction of movement, within the leading edge of the pll<sub>p</sub>, to one focusing towards actin-rich focal apical membranes within its trailing part. This dynamic tissue remodeling process is associated with enrichment of the apical junctional proteins ZO-1 and PRKC at apical focal points. Using a hair cell specific antibody, I could show that rosettes are organized around hair cell progenitor cells and are formed via the coordinated constriction of apical membrane of prospective support cells. Here I demonstrate that the cell polarity machinery, implicated previously in other cell types, is conserved also during pll<sub>p</sub> morphogenesis of the zebrafish. Loss of lethal giant larvae 2 (*Lgl2*), atypical protein kinase C iota (*aPKC<sub>i</sub>*) or Myosin VI causes lack of apical constriction, disruption of tissue organization, loss of polarity and failure of neuromast formation. Moreover, my study shows that *Lgl2* is not required for Notch/Delta expression. Therefore, failure of rosette formation, in the *lgl2* morphants, is not a consequence of a loss of hair-cell progenitor cells. In addition, Myosin II is implicated in control of pll<sub>p</sub> migration and its inhibition causes migratory defects of the pll<sub>p</sub>, which consequently result in a failure of neuromast deposition. Migratory defects, albeit weaker than caused by Myosin II inhibition, emerge also from the loss of *Lgl2*.



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# Zusammenfassung

Die Morphogenese von Gewebeverbänden, die Abänderung von Zellanordnungen innerhalb von Gewebeverbänden, sowie Zellwanderungen sind als wesentliche Kräfte an der Entwicklung von Organen beteiligt. Die Aufklärung der zellulären Maschinerie, welche der Umordnung von Zellen innerhalb von Gewebeverbänden und während der Organentwicklung zugrunde liegt, stellt ein wichtiges Problem der Entwicklungsbiologie dar.

Meine Arbeit beschäftigte sich mit der molekularen Charakterisierung von gewebsmorphogenetischen Prozessen in einem einfachen migratorischen Gewebeverband, dem Primordium des Laterallinienorgans des Zebrafisches *Danio rerio*. Während seiner Wanderung entlang der Mittelachse des Zebrafischembryos, lässt dieses Organ in regelmässigen Abständen Zellgruppen zurück, aus welchen in der weiteren Entwicklung die sensorischen Einheiten (Neuromasten) des Laterallinienorgans hervorgehen.

Ich konnte zeigen, dass es sich beim Primordium des Laterallinienorgans um ein migrierendes Epithelgewebe handelt. Innerhalb dieses Gewebes ordnen sich aktin-reiche apikale Adhäsionskomplexe von einer Anordnung, welche sich im Bereich der Migrationsfront orientiert entlang der Wanderungsrichtung des Primordiums, hin zu einer im posterioren Bereich, die fokussiert ist auf zentrale Punkte innerhalb zellulärer Rosetten. Dieser dynamische Prozess der Gewebeumgestaltung geht einher mit einer Anreicherung der apikalen Tight-junctions assoziierten Proteine Zonula occludens-1 (ZO-1) und der atypischen Protein kinase C (PRKC).

Mittels eines Haarzell-spezifischen Antikörpers konnte ich nachweisen, dass die Rosettenbildung durch koordinierte Konstriktion apikaler Membranen von Hilfszellen, sowie jeweils einer zentralen Haarvorläuferzelle, zustande kommt. Bei der Ausbildung von zellulären Rosetten fällt der Zellpolaritätsmaschinerie eine wichtige Rolle zu. Verlust der Proteine Lethal giant larvae 2 (Lgl2), PRKCiota, oder des nicht-konventionellen Myosin VI führt zum Verlust apikaler

Konstriktion, fehlerhafter Gewebeorganisation, Polaritätsverlust und zu einer Reduktion der Neuromastenentwicklung. Darüber hinaus konnte ich zeigen, dass Lgl2 nicht für die Delta Expression benötigt wird und dass somit die gestörte Rosettenbildung in *lgl2* Morphanten nicht auf einen Verlust von Haarzellvorläufern zurückzuführen ist.

In diesen Studien konnte ich schliesslich eine Funktion von Lgl2 in der gerichteten Zellwanderung des Primordiums beschreiben. Diese Defekte ähneln der Zellwanderungsstörung welche durch Inhibition von nicht-konventionellem MyosinII hervorgerufen wird.

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## List of publications

**Hava, D.**, Forster, U., B., Matsuda, M., Link, B., A., Eichhorst, J., Wiesner, B., Chitnis, A. and Abdelilah-Seyfried, S., Apical membrane maturation and cellular rosette formation during morphogenesis of the zebrafish lateral line (submitted)

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